FORMULATION AND OPTIMIZATION OF RAMIPRIL NIOSOMES

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MADURAI MEDICAL COLLEGE

MADURAI - 625020

Mrs. **R. THARABAI,** M.Pharm,
Professor and Head of the Department
Principal i/c,
Department of Pharmaceutical Chemistry,
College of Pharmacy,
Madurai Medical College,
Madurai– 625020.

CERTIFICATE

This is to certify that the dissertation entitled**, "Formulation and optimization of Ramipril Niosomes."** was done by **Ms. A.GOKILA** in the Department of Pharmaceutics, Madurai Medical College, Madurai – 20, in partial fulfillment of the requirement for the Degree of Master of Pharmacy in Pharmaceutics, is a bonafide work carried out by her, under the guidance and supervision of **Mr.A.Abdul Hasan Sathali**, **M.Pharm,** Professor and Head, in the Department of Pharmaceutics, during the academic year 2010 – 2011.

This dissertation is forwarded to the Controller of Examinations, The Tamil Nadu Dr. M.G.R. Medical University, Chennai.

Place : Madurai **(PROF. R. THARABAI)**

Date :

Mr. **A. Abdul Hasan Sathali,** M.Pharm,
Professor and Head of the Department,
Department of Pharmaceutics,
College of Pharmacy,
Madurai Medical College,
Madurai– 625020.

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This is to certify that the dissertation entitled**, "FORMULATION AND OPTIMIZATION OF RAMIPRIL NIOSOMES."** submitted by **Ms. A.Gokila,** in partial fulfillment of the requirement for the Degree of **Master of Pharmacy** in **Pharmaceutics**, is a bonafide work carried out by her, under my guidance and supervision during the academic year 2010 – 2011 in the Department of Pharmaceutics, Madurai Medical College, Madurai - 20.

I wish her success in all her endeavors.

Place : Madurai **(A. Abdul Hasan Sathali)**

Date :

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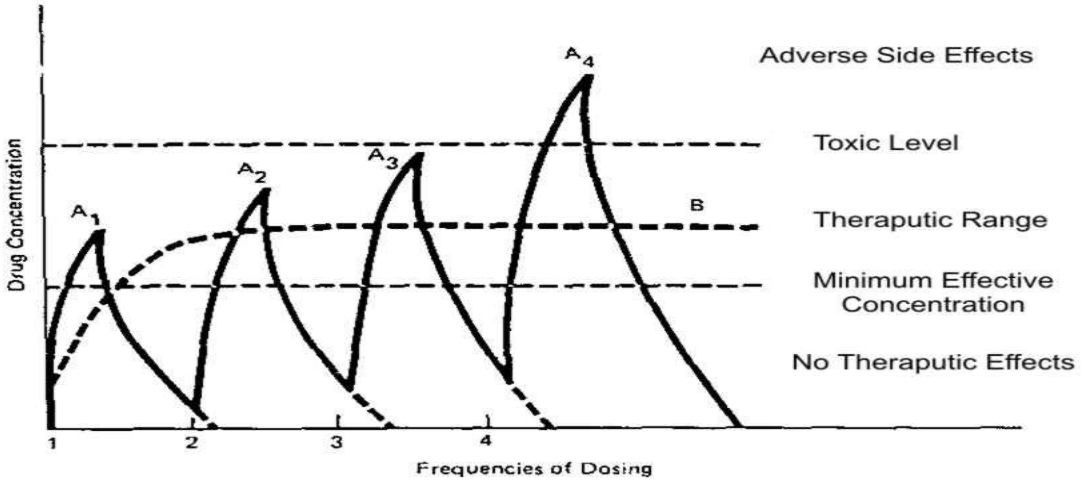
CONTENTS

CHAPTER – I

INTRODUCTION

For many decades treatment of an acute disease (or) a chronic illness has been mostly accomplished by delivery of drugs to patients using various pharmaceutical conventional dosage forms (such as tablets, capsules, pills, suppositories, creams, ointments, liquids, aerosols and injectables as drug carriers). [1] Even today conventional drug delivery system occupies most of the part in a prescription as well as drug store. This type of drug delivery system is known to provide a prompt release of drug.

Therefore, to achieve as well as to maintain the drug concentration within the therapeutically effective range needed for treatment, it is often necessary to take this type of drug delivery system several times a day. [1] This results in significant fluctuations in drug release. (Figure: 1) **Figure: 1**



Hypothetical drug concentration profiles in the systemic circulation resulting from the consecutive administration of multiple doses of an immediate-release drug delivery system (A_1, A_2, . . .) compared to the ideal drug concentration profile (B) required for treatment.

DISADVANTAGES OF CONVENTIONAL DOSAGE FORM [2]

1) Poor patient compliance- increased chances of missing the dose of a drug with short half life for which frequent administration is necessary.

2) A typical ***peak-valley*** plasma concentration-time profile is obtained which makes attainment of steady state condition is difficult.
3) The unavoidable fluctuations in the drug concentration may lead to ***under-medication*** or ***over-medication*** as the Css values fall or rise beyond the therapeutic range
4) The fluctuating drug levels may lead to precipitation of adverse effects especially of a drug with small therapeutic index whenever over-medication.

To overcome the above disadvantages, development of drug delivery systems capable of controlling the rate of drug delivery, sustaining the duration of therapeutic action and/or targeting the delivery of drug to a particular tissue [1].

They are as follows [3]

1. Delayed release.
2. Repeat action.
3. Prolonged release.
4. Sustained Release.
5. Extended release.
6. Controlled Release (Rate controlled).
7. Modified release.

1) Delayed Release

Delayed Release indicates the drug is not being released immediately following administration but at later time. Ex: enteric coated tablets; pulsatile release capsules.

2) Repeat action

Repeat action indicates that an indivual dose is released fairly soon after administration and second or third doses are subsequently at intermittent intervals.

3) Prolonged release

Prolonged release indicates that the drug is provided for absorption over a longer period of time than from a conventional dosage form. However there is an implication that onset is delayed because of an overall slower release rate from the dosage form.

4) Sustained Release

Sustained Release indicates an initial release of drug sufficient to provide a therapeutic dose soon after administration and then a gradual release over the extended period.

5) Extended Release

Sustained release dosage forms release drug slowly, so that plasma concentrations are maintained at a therapeutic level for a prolonged period of time. (Usually between 8 and 12 hours)

6) Controlled Release

Controlled release dosage forms release the drug at a constant rate, which is predictable and also the release rate is reproducible from one unit to another. It provide plasma concentrations that remain invariant with time.

7) Modified Release

Modified Release dosage forms are defined by the USP as those whose drug release characteristics of time course and for location are choosen to accompolish therapeutic or convenience objectives not offered by conventional forms whereas an extended release dosage form allows a 2 fold reduction in dosing frequency or increase in patient compliance

or therapeutic performance. It is interesting to note that the USP considers that the terms controlled release prolonged release and sustained release are interchangeable with extended release.

CLASSIFICATION OF CONTROLLED DRUG DELIVERY SYSTEMS (DDS) [2]

1. Rate-preprogrammed drug delivery systems.
2. Activation-modulated drug delivery systems.
3. Feedback-regulated drug delivery systems.
4. Site-targeting drug delivery systems.

TARGETED or SITE-SPECIFIC DDS:

Targeted DDS refers to systems that place the drug at or near the receptor site or site of action. Targeted drug delivery implies selective and effective localization of drug into the target(s) at therapeutic concentrations with limited access to target sites. [4]

A targeted drug delivery system is preferred in the following situations:

- **Pharmaceutical:** drug instability, low solubility.
- **Pharmacokinetic:** short half-life, large volume of distribution, poor absorption.
- **Pharmacodynamic:** low solubility, low therapeutic index.

Targeted drug delivery may provide maximum therapeutic activity by preventing drug degradation or inactivation during transit to the target sites. Meanwhile, it can protect the body from the adverse effects because of inappropriate disposition, and minimize toxicity of potent drugs by reducing dose. An ideal targeted delivery system should be nontoxic, biocompatible, biodegradable and physicochemically stable *in vivo* and *in vitro.* The

preparation of the delivery system must be reasonably simple, reproducible and cost-effective.

Site-targeted DDSs have also been characterized as-

- ***Passive targeting:*** refers to natural or passive disposition of a drug-carrier based on the physiochemical characteristics of the system in relation to the body.
- ***Active targeting:*** refers to alteration of the natural disposition of the drug carrier, directing it to specific cells, tissues or organs; for e.g. use of ligands or monoclonal antibodies which can target specific sites.
- ***Inverse targeting***
- ***Ligand mediated targeting***
- ***Physical targeting (Triggered release)***
- ***Dual targeting***
- ***Double targeting***
- ***Combination targeting*** [5]

Site-targeted DDS can be classified into three broad categories-

1. ***First-order targeting***: refers to DDS that delivers the drug to the capillary bed or the active site.
2. ***Second-order targeting***: refers to DDS that delivers the drug to a specific cell type such as the tumour cells and not to the normal cells.
3. ***Third-order targeting***: refers to DDS that delivers the drug intracellularly.

Drug targeting often requires ***carriers*** for selective delivery and can serve following purposes-

1. Protect the drug from degradation after administration.
2. Improve transport or delivery of drug to cells.

3. Decrease clearance of drug.
4. Combination of the above

Carriers for drug targeting are of two types-

- ***Carriers covalently bonded to drug:*** where the drug release is required for pharmacological activity.
- ***Carriers not covalently bonded to drug:*** where simple uncoating of the drug is required for pharmacological activity. E.g. liposomes.

The various carriers used for drug targeting are-

a. Polymeric carriers,
b. Albumin,
c. Lipoproteins,
d. Liposomes,
e. Niosomes,
f. Microspheres,
g. Nanoparticles,
h. Antibodies,
i. Cellular carriers and
j. Macromolecules.

COLLOIDAL DRUG CARRIERS: [6]

Colloidal drug delivery systems include micro- and nanoparticles, macromolecular complexes (e.g. lipoproteins), liposomes and niosomes. In many cases, colloidal carriers are used to improve stability of the drug either in biological fluids or in the formulation, to develop extended-release systems with targeting features and/or to enhance the therapeutic efficacy and reduce drug toxicity by modifying the distribution and controlling the disposition of the drug. [3]

CHAPTER – II

VESICULAR SYSTEMS –REVIEW

The quest never ends. From the very beginning of the human race; the quest is going on for newer and better alternatives; and in case of drugs it will continue; continue till we find a drug with maximum efficacy and no side effects. Many drugs, particularly chemotherapeutic agents, have narrow therapeutic window, and their clinical use is limited and compromised by dose limiting toxic effect. Thus, the therapeutic effectiveness of the existing drugs is improved by formulating them in an advantageous way.

NOVEL DRUG DELIVERY SYSTEM (NDDS) [7]

In the past few decades, considerable attention has been focussed on the development of new drug delivery system (NDDS). The NDDS should ideally fulfil two prerequisites.

- Firstly, it should deliver the drug at a rate directed by the needs of the body, over the period of the treatment.
- Secondly, it should channel the active entity to the site of action.

Conventional dosage forms including prolonged release dosage forms are unable to meet none of these. At present, no available drug delivery system behaves ideally, but sincere attempts have been made to achieve them through various novel approaches in drug delivery. [1]

Approaches are being adapted to achieve this goal, by paying considerable attention either to control the distribution of drug by incorporating it in a carrier system, or by altering the structure of the drug at the molecular level, or to control the input of the drug into the bioenvironment to ensure an appropriate profile of distribution.

NDDS aims at providing some control, whether this is of temporal or spatial nature, or both, of drug release in the body. Novel drug delivery attempts to either sustain drug action at a predetermined rate, or by maintaining a relatively constant, effective drug level in the body without concomitant minimization of undesirable side effects. It can also localize drug

action by spatial placement of controlled release systems adjacent to, or in the diseased tissue or organ; or target drug action by using carriers or chemical derivatization to deliver drug to particular cell type.

A number of NDDS have emerged encompassing various routes of administration, to achieve controlled and targeted drug delivery. Encapsulation of drug in vesicular structure is one such system, which can be predicted to prolong the existence of the drug in systemic circulation, and reduce the toxicity, if selective uptake can be achieved.

The vesicular systems are highly ordered assemblies of one or several concentric bilayers formed, when certain amphiphilic building blocks are confronted with water. Vesicles can be formed from a diverse range of amphiphilic building blocks. Biologic origin of these vesicles was first reported in 1965 by Bingham, and was given the name Bingham bodies.

COLLOIDAL DISPERSIONS:

The main therapeutic and commercial aims of colloidal drug carriers (CDC) include-

- Enhancement of oral bioavailability
- Decrease in variability and food dependency
- Development of i.v. injectable formulations
- Drug targeting to specific tissues (with reduction of general toxicity)
- Life cycle management (protection by proprietary formulation techniques).

VESICULAR SYSTEMS:

In recent years, vesicles have become the vehicle of choice in drug delivery. Lipid vesicles were found to be value of immunology, membrane biology, diagnostic techniques, and most recently, genetic engineering. Vesicles can play a major role in modelling biological membranes, and in the transport and targeting of active agents.

Biological membranes form the ubiquitous delimiting structures that surround and compartmentalize all cells and organelles. The bilayer arrangement of lipids is perhaps the only organizational feature that is common to all biological membranes. Numerous theoretical models of membrane structure have appeared since the publication of the cell

theory by Schleiden and Sehwann in 1839. Experimental models provide insight into the national dynamics and static structures of some isolated compartments of biological membranes. Lipid vesicles are just one type of many experimental models of biomembranes. Although developed for basic research, many technological innovations have arisen from the applications of these models. Lipid vesicles have evolved successfully, as vehicles for controlled drug delivery.

ADVANTAGES OF VESICULAR SYSTEMS: [8]

- Efficient method for delivery of drug directly to the site of infection.
- Reduction of drug toxicity with no adverse effects.
- Reduces the cost of the therapy by improved bioavailability of the medication,
- Incorporate both hydrophilic and lipophilic drugs.
- Delay drug elimination of rapidly meatbolizable drugs
- Function as sustained release systems.
- Solves the problems of drug insolubility, instability, and rapid degradation.

TYPES OF VESICULAR SYSTEMS:

Various types of vesicular systems are as follows,

- Liposomes
- Niosomes
- Transferosomes
- Pharmacosomes
- Enzymosomes
- Virosomes
- Ufasomes
- Cryptosomes
- Emulsomes
- Discomes
- Aquasomes
- Ethosomes
- Genosomes
- Photosomes
- Erythrosomes
- Hemosomes
- Proteosomes
- Vesosomes
- Archaeosomes
- Apsasomes
- Colloidosomes
- Cubasomes

LIPOSOMES: [2]

Liposomes (meaning ***lipid body***) are spherical microscopic vesicles compose of one or more concentric lipid bilayers, separated by water or aqueous buffer compartments with a diameter ranging from 25nm to 10000nm. Self assembly of phospholipid molecules in an aqueous environment results in the formation of liposomes [9].

They are commonly composed of one or more amphiphilic phospholipid bilayer membranes (and thus also called as *phospholipid vesicles)* that can entrap both hydrophilic and hydrophobic drugs. **Hydrophilic drugs are entrapped in the aqueous centre of liposome while the liposome wall, being phospholipid membrane can hold hydrophobic agents.** There are a number of components present in liposomes, with phospholipids and cholesterol being main ingredients. The phospholipids used for making liposomes include phosphatidylcholines (PC), phosphatidylethanolamines (PE) and phosphatidylserines (PS). Besides, phospholipids, sphingolipids, glycolipids and sterols can also be used to prepare liposomes. These vesicles are exploited to achieve altered drug pharmacokinetics and targeted therapies.

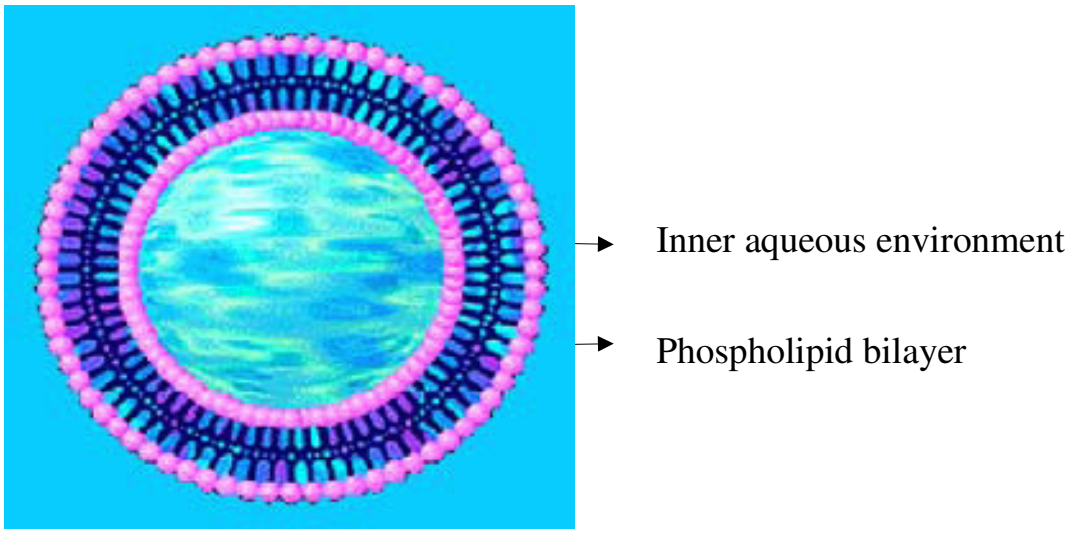


Figure: 2 Structure of liposomes.

CLASSIFICATION OF LIPOSOMES:

Depending upon their size and structure, liposomes are classified as follows,

1. MLV (multilamellar vesicles): These liposomes are made of series of concentric bilayers (5-20 layers) of lipids enclosing a small internal volume. They have a diameter of more than 5000nm.
2. OLV (oligolamellar vesicles): These are made of 2 to 5 bilayers of lipids surrounding a large internal volume. They have a diameter of 100- 1000nm.
3. MVV (multilvesicular vesicles): These have multicompartmental structures and have diameter more than 1000nm.
4. ULV (unilamellar vesicles): These are made of single bilayer of lipids. These may be further classified on the basis of their size into-
    - SUV (small unilamellar vesicles) of size 20 to 40nm.
    - MUV (medium unilamellar vesicles) of size 40 to 80nm.
    - LUV (large unilamellar vesicles) of size 100 to 1000nm.
    - GUV (giant unilamellar vesicles) of size greater than 1000nm.

Liposomes possess ***special characteristics*** such as-

1. Biodegradable and non-toxic.
2. Do not interact with drug or alter its activity.
3. Controlled hydration.
4. Control drug delivery rate: which in turn prevents degradation of drug, enhances their biological half-life and thus prolongs pharmacological effects. E.g. progesterone and cisplatin.
5. act as potent adjuvants to augment the immune response to recombinant protein vaccines. E.g. virosomes.
6. Ability to incorporate both water- and oil-soluble drugs.
7. Ability to protect labile compounds.
8. Available in a variety of sizes.
9. Serve as efficient solubilising vehicles for drugs with poor aqueous solubility such as alphaxalone, camptothecin, tacrolimus, econazole and paclitaxel.
10. Liposomes can be used for passive or active tissue targeting.

Disadvantages of liposomes:

- Liposomal formulations are expensive
- Liposomes are chemically unstable because of their predisposition to oxidative degradation.
- Tend to aggregate or lose entrapped drug during storage.
- Cannot be sterilizes by irradiation or by heat.
- Can be taken by the RES before reaching their target organ.
- High density lipoproteins tend to interact with liposomes *in vivo* leading to a loss of encapsulated species.
- Purity of natural phospholipids is another criterion for adoption of liposomes as drug delivery vehicles.

Due to the above limitations of liposomes newer vesicular system developed known as "Niosomes" using non- ionic surfactants.

NIOSOMES:

Niosomes are non-phospholipid vesicular alternative to liposomes. Niosomes are osmotically stable unilamellar or multilamellar vesicular systems obtained on hydration of synthetic non-ionic surfactants. The success achieved with liposomal systems stimulated the search for other vesicle-forming amphiphiles which led to the development of niosomes. Non-ionic surfactants were among the first alternative materials studied, and a large number of surfactants have since been found to self-assemble into closed bilayer vesicles that are used for drug delivery. [9]

TRANFEROSOMES [10]

Liposomal as well as niosomal systems are not suitable for transdermal delivery because of the poor skin permeability, breaking of vesicles, leakage of drug, aggregation and fusion of vesicles. To overcome these problems, a new type of carrier system called tranferosomes has recently being introduced which is capable of transdermal delivery of low as well as high molecular weight drugs.

Transferosomes are specially optimized, ultradeformable (ultraflexible), lipid supramolecular aggregates, which are able to penetrate the mammalian skin intact. Each transferosome consists of at least one inner aqueous compartment which is surrounded by a lipid bilayer with specially tailored properties, due to the incorporation of ‘‘**edge activators**’’ into the vesicular membrane. Surfactants such as sodium cholate, sodium deoxycholate, span 80 and tween 80 have been used as edge activators. It was suggested that transferosomes could respond to external stress by rapid shape transformations requiring low energy. These novel cariers are applied in the form of semidilute suspension without occlusion. Due to their deformability, transferosomes are good candidates for the noninvasive delivery of small, medium and large size drugs.

LIMITATIONS:

1. Transferosomes are chemically unstable because of the predisposition to oxidative degradation.
2. Lack of purity of the natural phospholipids comes in the way of adoption of transferosomes as drug delivery vehicles
3. Transferosomes formulations are expensive to prepare.

PHARMACOSOMES:

The limitations of tranferosomes as that of liposomes can be overcome by the pharmacosomes approach. These are defined as colloidal dispersions of drugs covalently bound to lipids and may exist as ultra fine vesicular, micellar (or) hexagonal aggregates,

depending on the chemical structure of drug – lipid complex. Many constraints of various classical vesicular drug delivery systems, such as problems of drug incorporation, leakage from the carrier, or insufficient shelf life can be avoided by the pharmacosome approach.

The idea for the development of the vesicular pharmacosome is based on surface and bulk interactions of lipids with drug. Any drug processing an active hydrogen atom (-COOH, -OH, $-NH_2$, etc) can be esterified to the lipid, with or without spacer chain. Synthesis of such a compound may be guided in such a way that strongly results in an amphiphilic compound, which will facilitate membrane, tissue or cell wall transfer, in the organism.

ENZYMOSOMES:

Liposomal construct engineered to provide a mini bioenvironment in which enzymes are covalently immobilized or couples to the surface of the liposomes. Used for targeted delivery to tumour cells.

VIROSOMES:

Liposomes spiked with virus glycoprotein, incorporated into the liposomal bilayers based on retro viruses derived lipids. Used for immunological adjuvants.

UFASOMES:

Vesicles enclosed by fatty acids obtained from long chain fatty acids (oleic acid, linoleic acid) by mechanical agitation of evaporated films in the presence of buffer solutions. Used for ligand mediated drug targeting.

CRYPTOSOMES:

Lipid vesicles with a surface coat composed of PC and of suitable polyoxyethylene derivative of phosphatidylethanolamine. Used for ligand mediated drug targeting.

EMULSOMES:

Nanosize lipid particles (bioadhesive nano emulsion) consisted of microscopic lipid assembly with apolar core which contains water insoluble drugs in the solution form without requiring any surface active agent or co solvent. These fat cored lipid paticles are dispersed in an aqueous phase. [13] Emulsome represents lipid based drug delivery systems with wide

range of therapeutic applications especially for parenteral delivery poorly water soluble drugs.

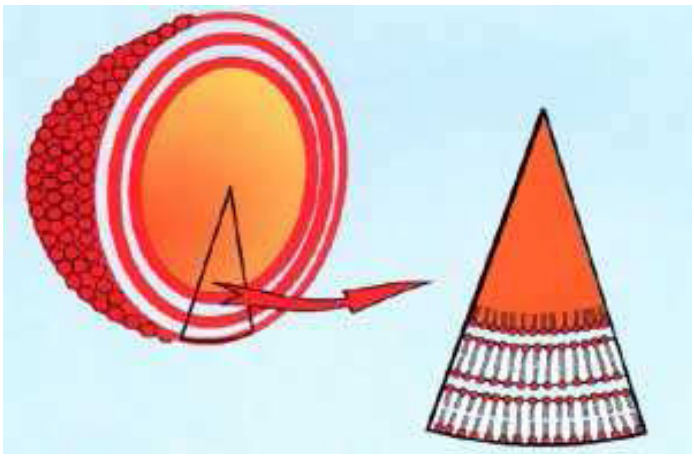


Figure 3: Diagrammatic structure of emulsome

DISCOMES:

Niosomes solubilised with non-ionic surfactant solution (Polyoxyethylene cetyl ether class, Solulan C_{24}). Discomes are large (12-60µm) structures and are capable of entrapping water-soluble solutes. [2] Used for ligand mediated drug targeting.

AQUASOMES:

Three layered self-assembly compositions with ceramic carbon nanocrystalline particulate core coated with glassy cellobiose. Used for specific targeting, molecular shielding.

ETHOSOMES:

Ethosomes are lipid "soft, malleable vesicles" embodying a permeation enhancer and composed of phospholipid, ethanol and water. Used for targeted delivery to deep skin layers.

GENOSOMES:

Artificial macromolecular complexes for functional gene transfer, cationic lipids are most suitable because they possess high biodegradability and stability in the blood stream. Used for cell specific gene transfer.

PHOTOSOMES:

Photolyase encapsulated in liposomes, which release the contents, by photo-trigerred charges in membrane permeability characteristics. Used for photo dynamic therapy.

ERYTHROSOMES:

Red blood cells offer a number of possibilities as drug carriers in controlled drug delivery systems. The release rate from erythrosomes, longevity and physical characteristics can be easily manipulated to alter the delivery mechanism and are used in both site-directed and sustained-release systems [9]. Liposomal systems in which chemically crosslinked human erythrocytes, cytoskeletons are used as a support to which lipid bilayer is coated. Used for effective targeting macromolecular drugs. An erythrocyte based drug carrier, 'Nanoerythrosome,' has been developed by extrusion of erythrocyte ghosts to produce small vesicles having an average diameter of 100 nm. Artificial red blood cells prepared by encapsulating haemoglobin by interfacial polymerization have been used as oxygen carriers.

HEMOSOMES:

Haemoglobin containing liposomes engineered by immobilizing haemoglobin with a polymerisable phospholipids. Used for high capacity oxygen carrying system.

PROTEOSOMES:

High molecular weight multi-subunit enzyme complexes with catalytic activity, which is specifically due to the assembly pattern of enzymes. Used for better catalytic activity turnover than non-associated enzymes.

VESOSOMES:

Nested bilayer compartments in vitro via the "interdigitated" bilayer phase formed by adding ethanol to a variety of saturated phospholipids. Application: Multiple compartments of the vesosome give better protection to the interior contents in serum.

ARCHAEOSOMES:

Vesicles composed of glycerolipids of archaea with potent adjuvant activity.

APSASOMES: [11]

Ascorbyl palmitate vesicles-Aspasomes.

Ascorbyl palmitate (ASP) was explored as bilayer vesicle forming material. Vesicles prepared with amphiphiles having antioxidant property may have potential applications towards disorders implicated with reactive oxygen species. Ascorbic acid (vitamin-C) is a

major antioxidant in human plasma as well as in and across cell membranes. It reduces α-tocopherol as well as peroxides and reactive oxygen species such as superoxide.

COLLOIDOSOMES: [8]

Colloidosomes is a novel class of microcapsules whose shell consists of coagulated or fused colloid particles at interface of emulsion droplets. The particles self assemble on the surface of droplets in order to minimize the total interfacial energy forming colloidosomes. Colloidosomes are the spherical capsules fabricated from the controlled self assembly of colloidal particles onto the emulsion droplets. For these colloidosomes, colloidal particles in aqueous solution adsorb onto the emulsion droplets in order to minimize the total interfacial energy and act as bridge between particles, locking them together and stabilizing the structure to allow removal of initial templating interfaces.

CUBASOMES: [12]

Cubosomes consist of honeycombed (cavernous) structures separating two internal aqueous channels and a large interfacial area. Self-assembled cubosomes as active drug delivery systems and they exhibit different internal cubic structure and composition with different drug-loading modalities. Cubosomes are nanoparticles whose size ranges from 10-500nm in diameter they appear like dots square shaped, slightly spherical.

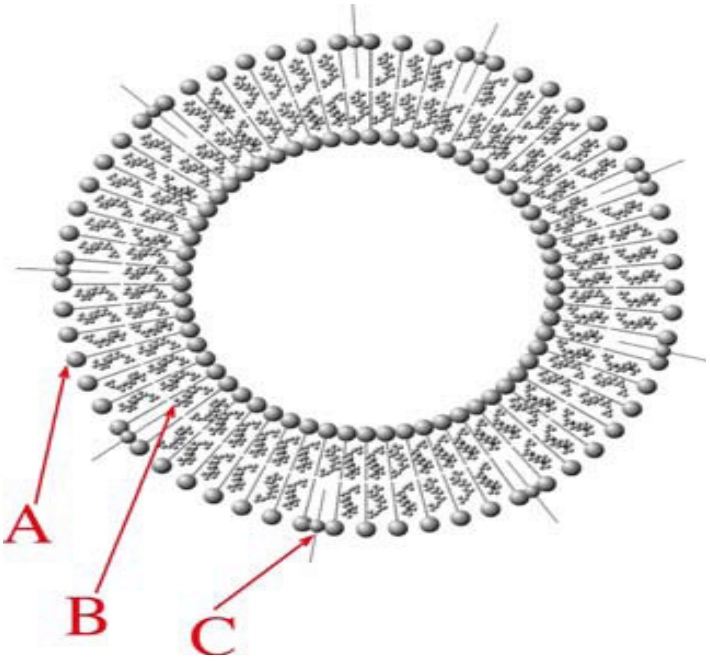
CHAPTER – III

NIOSOMES – REVIEW.

Niosomes are vesicles consisting of non-ionic surfactants. Niosomes were first reported by ***Vanler-berghe et al.*** *(*1972) and later by ***Handjani-Vila et al*** (1979) for their use in cosmetic industry [14]. Niosomes (Non- ionic surfactant vesicles- NSV) are now widely studied as alternative to liposomes (Baillie et al., 1985) [5]. From early 1980s, niosomes have gained wide attention by researchers for their use as drug targeting agents and drug carriers while avoiding demerits associated with the conventional form of drugs. These NSVs are widely used not only as models for cell membranes but also as drug carriers to deliver the drug into the targets of tumours and viruses.

Niosomes are essentially non-ionic surfactant based multilamellar or unilamellar vesicles in which an aqueous solution is entirely enclosed by a membrane resulted from the organization of surfactant macromolecules as bilayers [5]. Niosomes or Non- ionic surfactant vesicles are microscopic lamellar structures formed on admixture of non-ionic surfactant of alkyl or dialkyl polyglycerol ether class and cholesterol with subsequent hydration in aqueous media. The large number of available vesicle forming non-ionic surfactants makes these niosomes more attractive than liposomes for industrial production both in pharmaceutical and cosmetic applications.

a) 2-dimension



b) 3-dimension

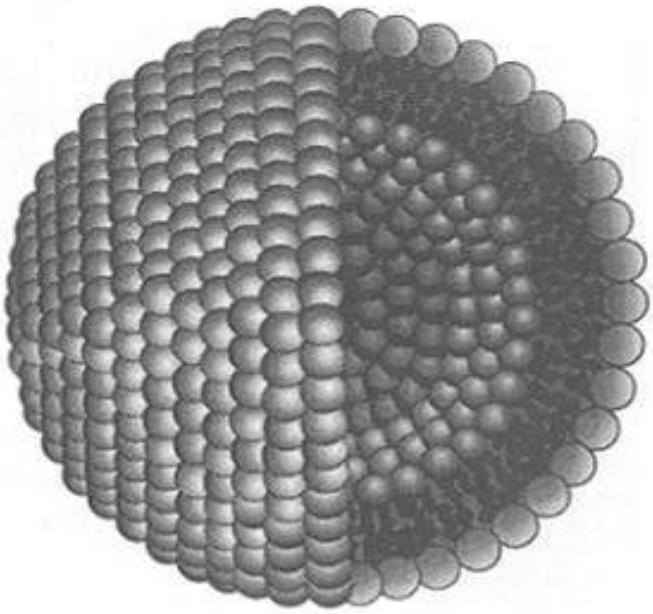


Figure 4: Structure of Niosomal vesicle a) in 2D *(A=span, B=cholesterol, C=dicetyl phosphate)*.

I. NATURE OF NIOSOMAL BILAYER [5, 7]

The bilayer membrane is an ordered structure and may exist in the gel state (or) liquid crystalline state. The gel state being most ordered structure and the liquid crystal state being less ordered. Addition of cholesterol abolishes the gel liquid transition state and makes the vesicle as in gel state (or) less leaky. So the nature of bilayer state depends on the type of lipid (or) surfactant and cholesterol.

II. VESICULAR COMPOSITION [7, 17]

Niosome consists of 2 components, which are the main component and membrane additives. The compositions of the main component are mainly non-ionic surfactants. Membrane additives are substances that are added in the formulation in order to stabilize the niosomes.

1. Main component : Non-ionic surfactant
2. Membrane additives:
    - Cholesterol
    - Stabilizer- Charged molecule
3. Drug

1. NON- IONIC SURFACTANTS [7]

Non-ionic surfactants are uncharged amphiphilic compounds. Like lipids the non-ionic surfactants also orient in an aqueous medium as planar bilayer lattices wherein polar (or) hydrophilic heads align facing aqueous bulk while hydrocarbon segments are so aligned that their interaction with aqueous media is minimized.

Every bilayer folds over itself to be a continuous membrane that forms vesicles so that hydrocarbon / water interface remains no more exposed.

Examples of non-ionic surfactants forming vesicles are, Polyoxy ethylene fatty acid esters, Polyoxy ethylene alkyl esters (including ethers of fatty alcohols) Polyoxy ethylene sorbitan esters, Polyoxy ethylene glyceryl mono and diesters, sucrose diester, Propylene glycol stearate, Long chain acyl amide, C_{12}-C_{22} fatty alcohols etc.,

BRIJ™ (Polyoxy ethylene fatty acid esters), SPAN™(Sorbitan fatty acid esters) and TWEEN™ (Polyoxy ethylene derivatives of sorbitan fatty acid esters.) are commercially available amphiphile surfactants.

The choice of non-ionic surfactant on vesicle formation depends on hydrophilic lipophilic balance (HLB), critical micellar concentration (CMC) and critical packing parameter of amphiphiles.

HLB of surfactants [15]

Hydrophilic lipophilic balance (HLB) is a good indicator of the vesicle forming ability of Surfactants.

- With the sorbitan ester (span) surfactants, a HLB number of between 4 and 8 was found to be compatible with vesicles formation.
- Tween 20 having HLB number 16.7 is to be too hydrophilic to form a bilayer membrane. However with an optimum level of cholesterol it forms niosomes.
- In addition to this ether amphiphiles bearing a steroidal C_{14} alkyl (or) C_{16} alkyl groups form vesicles.
- Polyoxy ethylene alkyl ether (Brij) forming vesicles increase six-fold bioavailability for (all surfactants that had HLB values from 8-14) intranasally administered insulin.

- Low phase transition temperature, increased leakage of low MW drugs from the aqueous compartment and decreased stability of the niosomes are the vesicular properties of hydrophilic surfactants.
- High phase transition temperature, decreased leakage of low MW drugs from the aqueous compartment and increased stability of the niosomes are the vesicular properties of hydrophobic surfactants.

CMC OF THE SURFACTANTS [15]

The CMC of the surfactants is the amount of free surfactant resulted that the presence of micelles does not influence the toxicity and the amount of free surfactant in the vesicle suspension is not primarily responsible for the toxic effects of the formulation.

CRITICAL PACKING PARAMETER [5, 15, 16]

The micelle-forming amphiphiles show relatively high solubility in water. The concentration corresponds to CMC in case of membrane forming lipids is significantly low. Unfortunately, the prediction of vesicle formation characteristics is not just a matter of HLB numbers, CMC values, it involves several other factors. ***Israelachvili*** (1991) suggested that parameters of self assemblages are governed by critical packing parameter (CPP). Their self-organization in water is mainly the result of the hydrophobic effect, as in the case of soap and detergent however it also depends on the relative proportions of hydrophobicity and hydrophilicity of the lipids as well as mesogen molecular geometry. The symmetry of the lipid self-assembly and liquid crystalline-phase formation show strong dependence on the molecular shape of the mesogen/amphiphiles. The different shapes and volumes constructing different phases are characterized by a dimensionless CPP.

The critical packing parameter (CPP) is defined as

$$\mathbf{CPP = v / l_c.a_o}$$

Where,

v = Hydrophobic group volume.

l_c = The critical hydrophobic group length

a_o = The area of hydrophilic head group

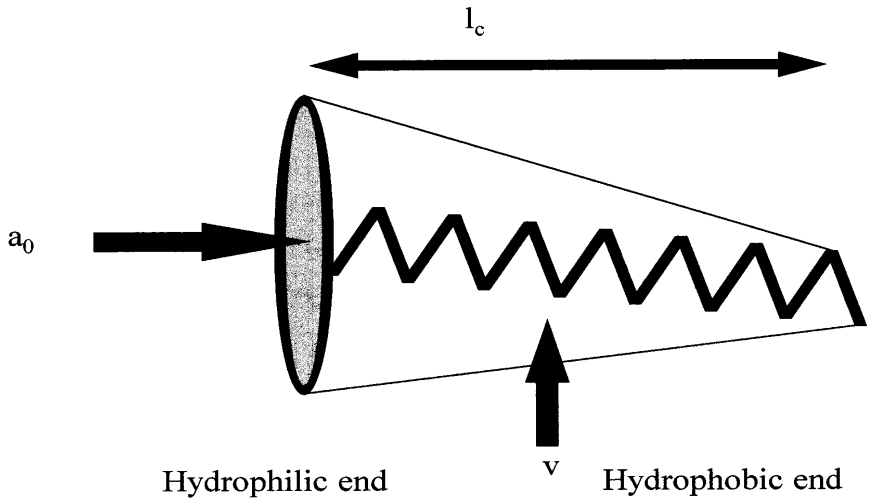


Figure 5: Schematic representation of an amphiphile.

The vesicle forming ability of amphiphiles depends on the CPP value which is determined by using hydrophobic group volume and area of hydrophilic head group.

CPP = 0.5-1 -- Surfactant is likely to form vesicles.

CPP < 0.5 -- Large hydrophilic head group give spherical micelles.

CPP > 0.5 -- Large contribution from the hydrophobic group value produces inverted micelles.

The different structures formed by amphiphilic molecules are represented in figure: 6.

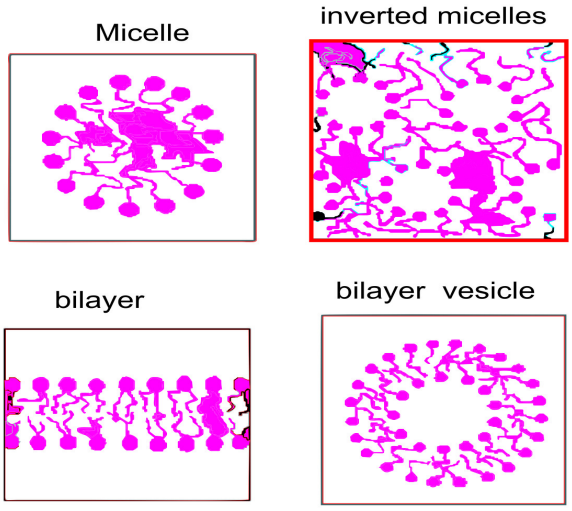


Figure 6: Examples of structures formed by amphiphilic molecules.

2. CHOLESTEROL [5, 16, 17]

Steroids are important components of cell membrane bring bilayer fluidity and permeability. The most **common additive** found in niosomal systems is cholesterol which is known to *abolish the gel to liquid phase transition* of liposomal and niosomal systems, resulting in less leakiness of the vesicles. However, it may have effects on membrane permeability, encapsulation efficiency, bilayer rigidity, ease of rehydration of freeze dried niosomes and toxicity. In general, it has been found that a molar ratio of 1:1 between cholesterol and non-ionic surfactants is an optimal ratio for the formulation of physically stable niosomal vesicles Cholesterol can be incorporated in bilayers at significantly higher molar ratio, however by itself does not form niosomal bilayer. Its -OH group orients towards aqueous phase while aliphatic chains parallel to the hydrocarbon chain of surfactants.

Cholesterol is known to have important modulatory effect on the bilayer membrane. Cholesterol acts as '*fluidity buffer'*, since below the phase transition it tend to make the membrane less ordered while above the transition it tends to make the membrane more ordered, thus suppressing the tilts and shift in membrane structure specifically at the phase transition.

Role of cholesterol in bilayer formation:

- Acts as a fluidity buffer.
- After intercalation with phospholipid molecules alters the freedom of motion of carbon molecules in the acyl chain.
- Restricts the transformations of *trans- to gauche-* conformations.

3. DRUG [16]

The drug is actively or passively entrapped in vesicles. In passive trapping, drug and lipids are co dispersed with fraction of drug being entrapped, according to hydrophobicity and electrostatic charge. If the drug is hydrophilic, it will be entrapped in the internal aqueous phase and the hydrophobic drug will be entrapped in lipid region. Active trapping can be achieved by ion gradients placed across the niosomal membranes. This allows drug entrapment after the niosomal carrier has been formulated.

The physico-chemical properties of encapsulated drug influence charge and rigidity of the niosome bilayer. The drug interacts with surfactant head groups and develops the charge that creates mutual repulsion between surfactant bilayers and hence increases vesicle size. The aggregation of vesicles is prevented due to the charge development on bilayer.

4. **STABILIZER** [5, 16, 27]

One of the methods used to stabilize niosomes is to add a charged molecule to the bilayer. Used for preventing aggregation of niosomes. [17]

- Negatively charged molecules - Dicetylphosphate (DCP),
  - Phosphatidic acid.
- Positively charged molecules - Stearylamine (STR),
  -Cetylpyridinium chloride.
- Non-ionic substances - Cholesteryl poly-24-oxyethylene ether (SC24)

Normally, the charged molecule is added in niosomal formulation in an amount of 2.5–5 mol% because the high concentration of charged molecules can inhibit the formation of niosomes. It is added in niosomal Stable niosomal dispersion must exhibit a constant particle size and a constant level of entrapped drug. There must be no precipitation of the membrane components.

The inclusion of a charged molecule in the bilayer shifts the electrophoretic mobility making it positive or negative and prevents niosomes aggregation.

Examples of positive charge inducers (11, 12)

Protamine, Polyamine, Polyvinyl pyridine, Poly oxethane, Poly amidoamines, Cetyl pyridinium chloride, Stearyl amine, Diethanolamine etc.,

Examples of negative charge inducers (11, 12)

Oleic acid, Palmitic acid, Dicetyl phosphate, Cetyl sulphate, Phosphatidic acid, Phosphatidyl serine etc,

The amount of charge modifying compound ranges from 0.01 to 0.5-wt%

III. TYPES OF NIOSOMES [7]

They are divided in to three types

They are as follows,

1. Multilamellar niosomes (>0.05µm)
2. Small unilamellar niosomes (0.025-0.05µm)
3. Large unilamellar niosomes (>0.01µm)

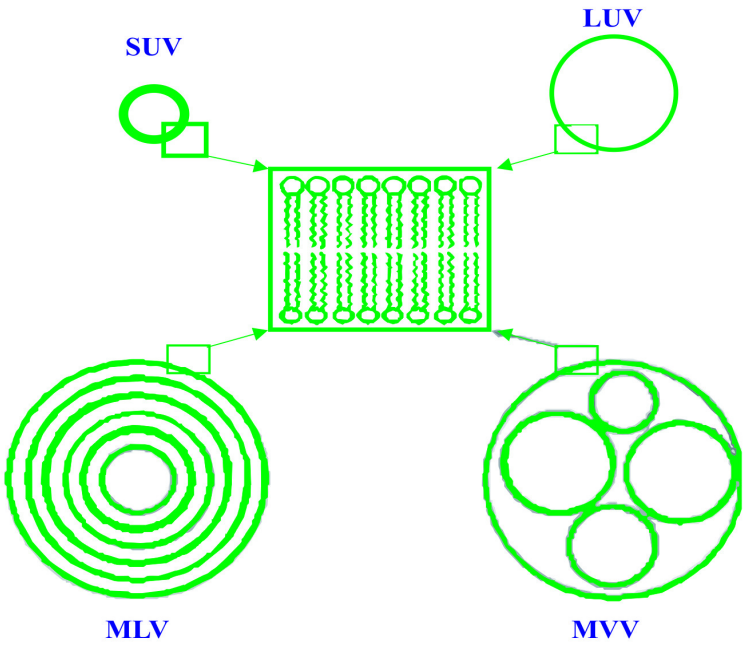


Figure 7: Schematic illustration of different size and number of lamellae

SUV: Small unilamellar vesicles **LUV:** Large unilamellar vesicles

MLV: Multilamellar vesicles**, MVV:** Multi vesicular vesicles.

IV.METHODS OF PREPARATION OF NIOSOMES [5, 7, 15, 18,19, 10, 11, 12, 13, 17, 18, 19, 20, 21]

1. **Ether Injection Method**
2. **Hand Shaking Method (Thin film hydration technique)**
3. **Sonication**
4. **Microfludization**

5. **Multiple Membrane Extrusion method**
6. **Reverse Phase Evaporation Technique (REV)**
7. **Trans membrane pH gradient (inside acidic) Drug Uptake Process (remote Loading**
8. **The "Bubble" Method**
9. **Ethanol injection method**
10. **Formation of niosomes from proniosomes**
11. **Emulsion method**
12. **Lipid injection method**
13. **Niosome preparation using micelle**

1. Ether Injection Method [5, 15, 18, 19]

This method provides a meaning of making niosomes by slowly injecting the surfactant/cholesterol mixture (dissolved in diethyl ether) in to the aqueous phase maintained at 60^0 through 14-gauge needle. This method produces unilamellar vesicle shows highest entrapment efficiency. Depending upon the conditions used, the diameter of the vesicles range from 50-1000nm.

2. Hand Shaking Method (Thin film hydration technique) [5, 15, 18,22]

The mixture of vesicles forming ingredients like surfactant and cholesterol are dissolved in a volatile organic solvent (diethyl ether, chloroform or methanol) in round bottom flak. The solvent is evaporated at a temperature (20^0C) using a rotary evaporator, leaving a thin layer of solid mixture deposited on the wall of the flask. The dried surfactant film can be rehydrated with aqueous phase maintained at 0-60º C with gentle agitation.

The apparatus used for thin film hydration method and the mechanism of formation of niosomes is shown in the **Figure.8a & 8b.**

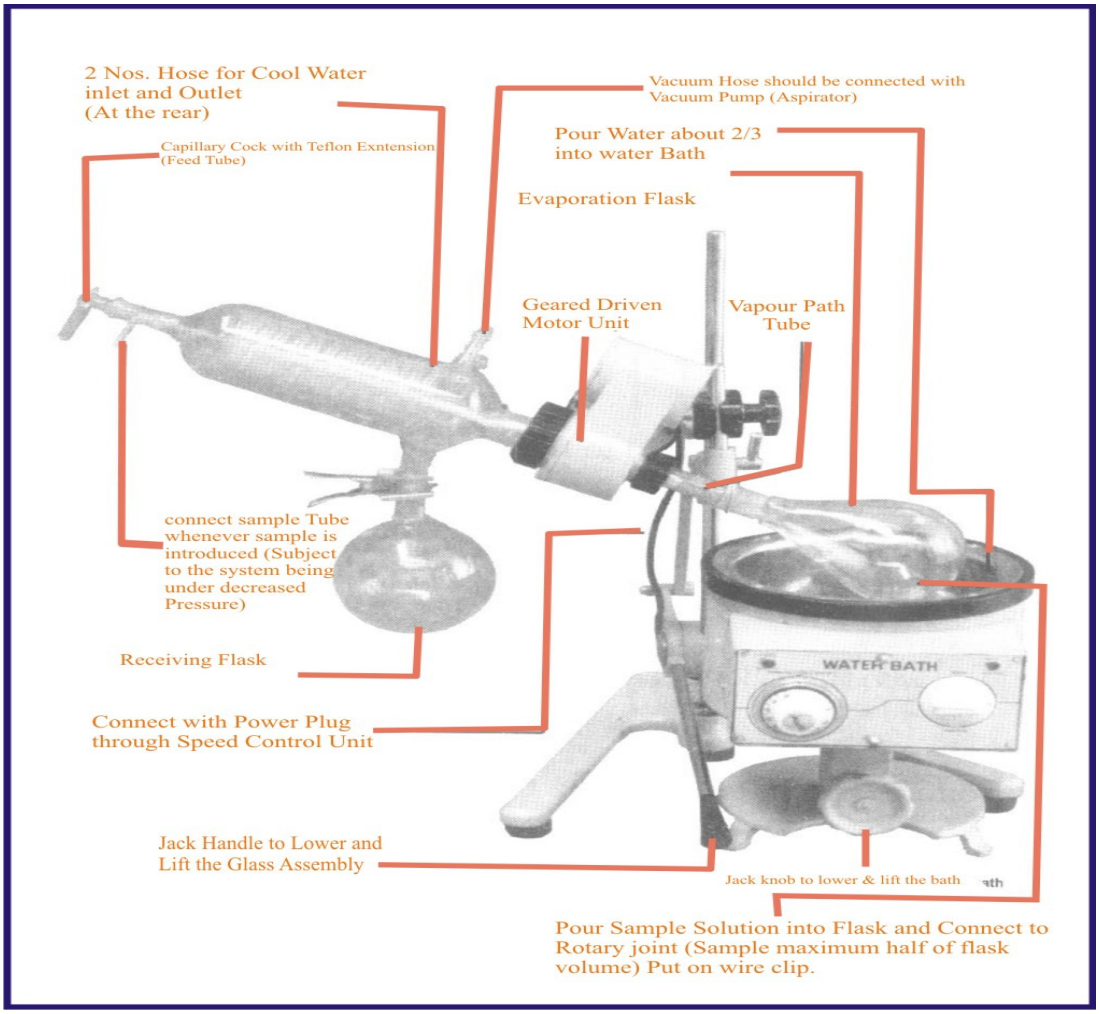


Figure-8a: Rotary Flash Evaporator.

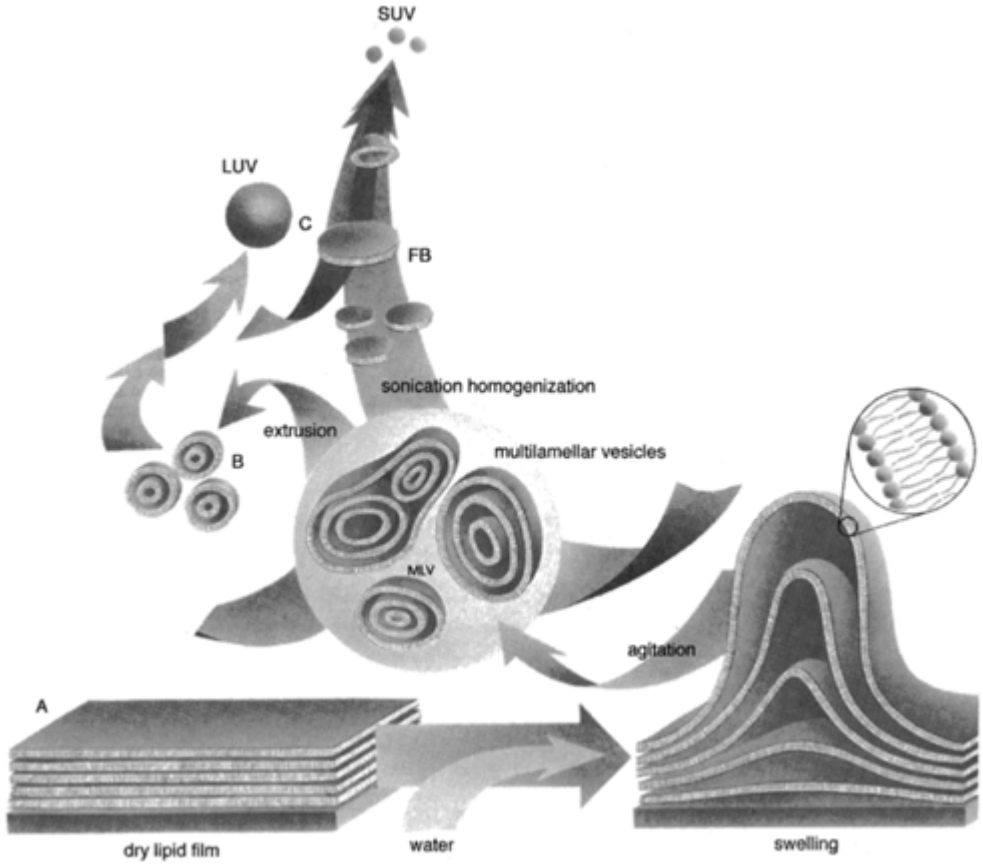


Figure 8b: Possible formation of niosome vesicles when hydrating in aqueous solution. [5, 33]

Mechanism of Niosome Formation:

The lipids are casted as stacks of film from their organic solution using rotary flash evaporator under reduced pressure. And then the casted film is dispersed in an aqueous medium upon hydration the lipids (surfactants and cholesterol) **swell and peel off** from the wall of the round bottom flask and **vesiculate** forming multilamellar vesicles. The mechanical energy required for the swelling of the lipids and dispersion of casted lipid film is imparted by manual agitation.

Thermo sensitive niosomes are prepared at 60º C by evaporating organic solvent and leaving a thin film of lipid of on the wall of rotary flask evaporator. The aqueous phase containing drug is added slowly by shaking at room temperature followed by sonication.

3. Sonication [14, 22, 34, 35]

The surfactant/cholesterol mixture in organic solvent is mixed with aqueous phase in a vial. Then the mixture is probe or bath sonicated at 60^0 for 3minutes to produce niosomes. The vesicles produced are unilamellar and smallest in size.

4. Micro fludization [18, 22, 35]

Microfludization is the recent technique used to prepare unilamellar vesicles of a defined size distribution. This method is based on submerged jet principle in which two fluidized streams interact at ultra high velocities (up to 1700 ft/sec) in precisely defined micro channels within the interaction chamber. The impingement of thin liquid sheet along a common front is arranged such that the energy supplied to the system remains within the area of niosomes formation. The result is a greater uniformity, smaller size and better reproducibility of niosomes formed (Mayer *et al*., 1985).

5. Multiple Membrane Extrusion method [18, 22]

Mixture of surfactant, cholesterol and dicetyl phosphate in chloroform is made into thin film by evaporation. The film is hydrated with aqueous drug solution and the resultant suspension is extruded through polycarbonate membranes which are placed in series up to 8 passages. It is a good method for controlling niosomes size. (Mayer *et al*., 1985).

6. Reverse Phase Evaporation Technique (REV) [18, 21, 22]

The novel key in this method is the removal of solvent from an emulsion by evaporation. Cholesterol and surfactant (1:1) are dissolved in a mixture of ether and chloroform. An aqueous phase containing drug is added to this and the resulting two phase system is sonicated at 4-5°C. The clear gel formed is further sonicated after the addition of a small amount of phosphate buffered saline (PBS). The organic phase is removed at 40°C under low pressure. The resulting viscous niosome suspension is diluted with PBS and heated on a water bath at 60°C for 10 minutes to yield niosomes.

7. Trans membrane pH gradient (inside acidic) Drug Uptake Process (remote Loading) [18, 22, 35]

Surfactant and cholesterol are dissolved in chloroform. The solvent is then evaporated under reduced pressure to get a thin film on the wall of the round bottom flask. The film is hydrated with 300 mM citric acid (pH 4.0) by vortex mixing. The resulting multilamellar vesicles are then treated to three freeze thaw cycles and sonicated. To this niosomal suspension, aqueous solutions containing 10 mg/ml of drug is added and vortexed. The pH of the sample is then raised to 7.0-7.2 with the addition of 1M disodium phosphate (this causes the drug which is outside the vesicle to become non-ionic and can then cross the niosomal membrane, and once inside it is again ionized thus not allowing it to exit the vesicle). This mixture is later heated at 60°C for 10 minutes to give niosomes.

8. The "Bubble" Method [18, 22, 35]

It is novel technique for the preparation of liposomes and niosomes without the use of organic solvents. The bubbling unit consists of round-bottomed flask with three necks positioned in water bath to control the temperature. Water-cooled reflux and thermometer is positioned in the first and second neck and nitrogen supply through the third neck. Cholesterol and surfactant are dispersed together in this buffer (pH 7.4) at 70°C, the dispersion mixed for 15 seconds with high shear homogenizer and immediately afterwards "bubbled" at 70°C using nitrogen gas.

9. Ethanol injection method [21]

This method has been reported as one of the alternatives used for the preparation of small unilamellar vesicles (SUVs) without sonication. In this method, an ethanol solution of surfactant is injected rapidly through a fine needle into excess of saline or other aqueous

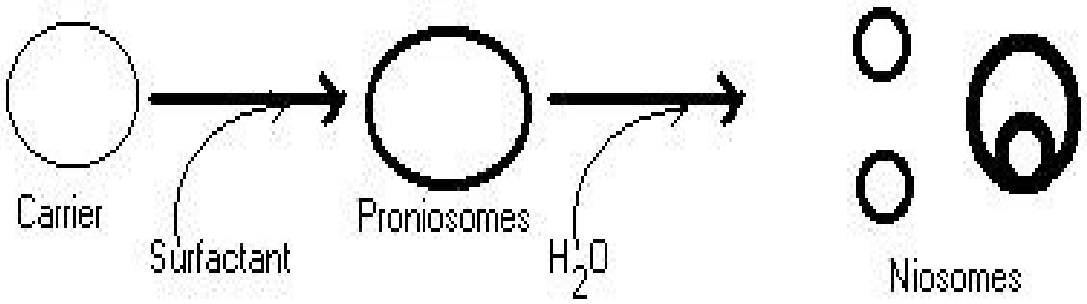
medium. Vaporization of ethanol leads to the formation of vesicles. Fang et al entrapped enoxacin in niosomes using this particular method. [22]

10. Formation of niosomes from proniosomes [5, 18, 22, 29, 30, 31]

Another method of producing niosomes is to coat a water-soluble carrier such as sorbitol with surfactant. The result of the coating process is a dry formulation. In which each water-soluble particle is covered with a thin film of dry surfactant. This preparation termed as "proniosomes". The niosomes are recognized by the addition of aqueous phase as **T > Tm** and brief agitation.

T – Temperature

Tm – Mean phase transition temperature



Blazek-Walsh A.I. *et al* [20] have reported the formulation of niosomes from maltodextrin based proniosomes. This provides rapid reconstitution of niosomes with minimal residual carrier. Slurry of maltodextrin and surfactant was dried to form a free flowing powder, which could be rehydrated by addition of warm water.

11. Emulsion method [20]

The oil in water emulsion is prepared from an organic solution of surfactant, cholesterol, and an aqueous solution of the drug. The organic solvent is then evaporated, leaving niosomes dispersed in the aqueous phase.

12. Lipid injection method [20]

This method does not require expensive organic phase. Here, the mixture of lipids and surfactant is first melted and then injected into a highly agitated, heated aqueous phase containing dissolved drug. Here, the drug can be dissolved in molten lipid and the mixture will be injected into agitated, heated aqueous phase containing surfactant.

13. Niosome preparation using micelle [9]

Niosomes may also be formed from a mixed micellar solution by the use of enzymes. A mixed micellar solution of C16G2, dicalcium hydrogen phosphate, polyoxyethylene cholesteryl sebacetate diester (PCSD) converts to a niosome dispersion when incubated with esterases. PCSD is cleaved by the esterases to yield polyoxyethylene, sebacic acid and cholesterol. Cholesterol in combination with C16G2 and DCP then yields C16G2 niosomes.

V. THE REDUCTION OF NIOSOME SIZE [5]

A reduction in vesicle size may be achieved by a number of

- Probe sonication
- Extrusion through 100nm nucleopore filter
- The combination of sonication and filtration

- Micro fluidizer
- High pressure homogenizer.

VI. SEPERATION OF UNENTRAPPED DRUG [15, 18, 27]

In vesicular system half of the drug is encapsulated and half is external to the niosomes may eventually yielding systems with a beneficial biphasic biodistribution profile. The removal of unentrapped solute from the vesicles can be accomplished by various techniques, which include: -

1. Dialysis

The aqueous niosomal dispersion is dialyzed in dialysis tubing against phosphate buffer or normal saline or glucose solution.

2. Gel Filtration

The unentrapped drug is removed by gel filtration of niosomal dispersion through a Sephadex-G-50 column and elution with phosphate buffered saline or normal saline.

3. Centrifugation

The niosomal suspension is centrifuged and the supernatant is separated. The pellet is washed and then resuspended to obtain a niosomal suspension free from unentrapped drug.

VII. CHARACTERIZATION OF NIOSOMES [7, 20, 21]

1. Entrapment efficiency

After preparing niosomal dispersion, unentrapped drug is separated by dialysis, centrifugation or gel filtration as described above and the drug remained entrapped in niosomes is determined by complete vesicle disruption using 50% n-propanol or 0.1% Triton X-100 and analyzing the resultant solution by appropriate assay method for the drug. Where,

(Total drug – Drug in supernatant)

$$\% \text{ drug entrapment} = \frac{\text{Total drug}}{\text{Total drug}} \times 100.$$

2. Vesicle diameter

Niosomes, similar to liposomes, assume spherical shape and so their diameter can be determined using light microscopy, photon correlation microscopy, and Scanning electron microscopy and freeze fracture electron microscopy.

3. In-vitro release

A method of *in-vitro* release rate study includes the use of dialysis tubing. A dialysis sac is washed and soaked in distilled water. The vesicle suspension is pipetted into a bag made up of the tubing and sealed. The bag containing the vesicles is placed in 200 ml of buffer solution in a 250 ml beaker with constant shaking at 25°C or 37°C. At various time intervals, the buffer is analyzed for the drug content by an appropriate assay method.

VIII. FACTORS AFFECTING VESICLES SIZE, ENTRAPMENT EFFICIENCY AND RELEASE CHARACTERISTICS [7, 9, 11]

1. Drug

Entrapment of drug in niosomes increases vesicle size, probably by interaction of solute with surfactant head groups and mutual repulsion of the surfactant bilayers. The hydrophilic lipophilic balance of the drug also affects degree of entrapment.

2. Amount and type of surfactant

The mean size of niosomes increases proportionally with increase in the HLB of surfactants like Span 85 (HLB 1.8) to Span 20 (HLB 8.6) because the surface free energy decreases with an increase in hydrophobicity of surfactant.

3. Cholesterol content and charge

Inclusion of cholesterol in niosomes increases its hydrodynamic diameter and entrapment efficiency. An increase in cholesterol content of the bilayers resulted in a decrease in the release rate of encapsulated material and therefore an increase of the rigidity of the bilayers obtained.

Presence of charge tends to increase the interlamellar distance between successive bilayers in multilamellar vesicle structure and leads to greater overall entrapped volume.

4. Methods of preparation

Hand shaking method forms vesicles with greater diameter (0.35-1μm) compared to the ether injection method (50-1000nm). Small sized niosomes can be produced by Reverse Phase Evaporation (REV) method. Microfludization method gives greater uniformity and small size vesicles.

Parthasarthi *et al* prepared niosomes by transmembrane pH gradient (inside acidic) drug uptake process. Niosomes obtained by this method showed greater entrapment efficiency and better retention of drug

5. Resistance to osmotic stress

Addition of a hypertonic salt solution to a suspension of niosomes brings about reduction in diameter. In hypotonic salt solution, there is initial slow release with slight swelling of vesicles probably due to inhibition of eluting fluid from vesicles followed by faster release which may be due to mechanical loosening of vesicles structure under osmotic stress.

IX. ROUTES OF ADMINISTRATION[5]

Azmin et al., investigated niosomal delivery of methotrexate to mice by oral and intravenous administration. On oral administration absorption of methotrexate was significantly increased.

Hofland et al., studied transdermal delivery of estradiol entrapped within niosomes. **Brewer and Alexander** performed studies on adjuvant activity of niosomes on the BALB/C humoral response to bovine serum albumin after intraperitoneal and subcutaneous administration.

X.IN VIVO BEHAVIOUR OF NIOSOMES [5]

In vivo niosomes have been found equiactive in liposomes in improving the therapeutic performance of drug and their distribution in the body .Like liposomes, niosomes are taken up by the liver and break down to release the free drug which re enters the circulation and maintains the plasma drug level.

Parthasarathi et al found niosomes to be stable in plasma. **Moser et al** found niosomes bearing hemoglobin to be physically stable and albumin, transferrin were absorbed on vesicles without destabilizing them.

XI. CHARACTERIZATION OF NIOSOMES: [14, 21, 34]

Table no 1: Analytical methods for characterizing niosomes.

S.NO.	PARAMETER(S)	METHOD(S)
1.	Morphology	Transmisson Electron Microscopy, Scanning Electron Microscopy, Optical Microscopy(OM), Cryo- Scanning Electron Microscopy, Freeze Fracture Microscopy.
2.	Vesicle size determination and Size distribution	Dynamic Light Scattering using particle Size Analyzer(PSA), Malvern Master Sizer, Photon Correlation Spectroscopy (PCS), OM, SEM, Laser Diffraction PSA.

3.	Zeta potential/ Surface Charge	Micro-electrophoresis meter, High Performance Capillary electrophoresis and Malvern Zeta Sizer (Zetameter)
4.	Rheological Properties (Elasticity)	Ostwalt U-tube, Low shear Rheo Analyzer & Extrusion method.
5.	Micro viscosity of niosomal membrane	Spectroflurophotometer.
6.	Viscosity	Ostwald's viscometer
7.	Membrane micro-structure	Negative Staining TEM.
8.	Lamellarity	OM, TEM
9.	Bilayer spacing and thickness	X-Ray Scattering Analysis.
10.	Gel-Liquid transition temperature & Thermal Analysis	Differential Scanning calorimetry, Differential Thermal Analysis, & Hot Stage Microscopy.
11.	Circular Dichroism	Spectropolarimeter.
12.	Micropolarity measurement	Fluorescence Spectrophotometer.
13.	Fluidity of vesicles	Differential Polarized Phase Fluorimetry
14.	Turbidity measurement	UV-Visible Diode Array Spectrophotometer.
15.	Entrapment Efficiency	Centrifugation method, Dialysis method, Gel Exclusion Chromatography.
16.	In-vitro release rate	Using dialysis membrane.

17.	Permeation study	Franz Diffusion Cell.
18.	Conductivity	Conductometer

XII.TOXICITY AND STABILITY [5]

Non-ionic surfactants used in niosomes are non-toxic and no toxic effects have been reported so far in animal studies due to the use of niosomes as drug carriers.

Jain et al didn't observe any morphological changes on storage for three months. **Baille et al** determined the stability in buffer and reported that the amount of entrapped solute would be retained under long term storage conditions.

XIII. ADVANTAGES OF NIOSOMES [22, 24, 27, 28]

- Biodegradable, biocompatible and non-immunogenic.
- Can entrap a wide range of compounds ranging from small soluble ions to complex macromolecules.
- They possess a great deal of flexibility in their structural characteristics.
- Niosomes can entrap both water-soluble and oil soluble drugs.
- They can be made to reach the site of action by oral, parenteral and as well as by topical route.
- Enhance the skin penetration of drugs.
- Prolong the circulation of entrapped drugs.
- Due to the presence of better targeting nature it is proved that usage of niosomal technology in treating cancer, parasitic, viral and other microbial diseases are more effective.
- Reduces systemic toxicity of drugs such as anti-cancer, anti-infective etc.,

- As a carrier for drug delivery to specific cells, they improve the therapeutic index of drug by restricting its effects to target cells.

XIV.STUDIES ON NIOSOMES AND THEIR MEDICINAL APPLICATIONS [14, 34]

Table no: 2.

S.NO.	PURPOSE / APPLICATION	DRUGS STUDIED
1.	Cancer chemotherapy and targeted drug delivery	Doxorubicin, Danorubicin Hcl, Methotrexate (MTX), 5-flurouracil, Adiramycin, Vincristine, Cytarabine Hcl
2.	Transdermal drug delivery	Nimesulide, Lidocaine, Cyclosporine, Estradiol, Erythromycin, α-interferon, Indomethacin, Enoxacin, Finasteride.
3.	Enhancement of bioavailability	Diclofenac, Flurbiprofen, Bleomycin, Vincristine, Doxorubicin, Acetazolamide.

4.	Ocular drug delivery	Timolol maleate, Acetazolamide, Cyclopetolate.
5.	Pulmonary drug delivery	All trans retinoic acid (ATRA).
6.	Brain Targeted Drug Delivery	VIP loaded glucose bearing niosomes.
7.	Protein/Peptide and Hormone delivery	LHRH, Insulin (oral), 9-desglycinamide -8-arginine vasopressin (DGAVP).
8.	Local/Intra Articular drug delivery	Radiolabelled Diclofenac Na niosomal vesicles.
9.	Enhancement of stability improved photostability	DGAVP, Haemoglobin, Dithranol, β-carotene
10.	Improved thermal and oxidative stability	β-carotene
11.	Prolonged release	Propranolol Hcl, Doxorubicin.
12.	For improved anti-infective therapy	Sodium stibogluconate, Rifampicin.
13.	Immuno stimulatory niosomes (antigenic)	Haemagglutinin, Ovalbumin, Hepatitis B DNA vaccine niosomes, Plasmid DNA encoding proteins of Hepatitis B virus, Influenza DNA vaccine niosomes, and Tetanus Toxoid Niosomes.
14.	Diagnosis	Urokinase

15.	Radio-pharmaceutical carrier and imaging study.	Iobitridol (X-ray imaging studies), and Iopromide (Kidney imaging studies)

OTHER APPLICATIONS

(a) Sustained release

Niosomes can provide relatively constant and sustained blood stream level of drug concentration. Sustained release action of niosomes could be applied to drugs with low therapeutic index since these could be maintained in the circulation via niosomal encapsulation.

(b) Localized drug action

Localization of drug action results in enhancement of efficacy or potency of the drug and at the same time reduces its systemic toxic effects. Niosomes are a promising vehicle for drug delivery and being non-ionic it is less toxic and improves the therapeutic index of drug by restricting its action to target cells.

ENHANCEMENT OF BIOAVAILABILITY (LIPOPHILICITY) [2]

The lipophilic form of drug has enhanced membrane/water partition coefficient as compared to the hydrophilic form of the drug. A big advantage of increased bioavailability through increased lipophilicity is reduction in drug dosage.

CHAPTER – IV

LITERATURE REVIEW.

1. Mahmoud Mokhtar et a1., studied the effect of some formulation parameters on flurbiprofen encapsulation and release rates of niosomes prepared from proniosomes. Proniosomal gels or solutions of flurbiprofen were developed based on span 20 , span 40, span 60, and span 80 without and with cholesterol. Niosomes formed immediately upon hydrating proniosomal formulae. The entrapment efficiency (EE%) of flurbiprofen (a poorly soluble drug) was either determined by exhaustive dialysis of freshly prepared niosomes or centrifugation of freeze-thawed vesicles. The influence of different processing and formulation variables such as surfactant chain length, cholesterol content, drug concentration, total lipid concentration, negatively or positively charging lipids, and the pH of the dispersion medium on flurbiprofen EE% was demonstrated. Results indicated that the EE% followed the trend Sp 60 (C18)>Sp 40 (C16)>Sp 20 (C12)>Sp 80 (C18). Cholesterol increased or decreased the EE% depending on either the type of the surfactant or its concentration within the formulae.

2. Toshimitsu Yoshioka et al., studied the formation of niosomes with a series of sorbitan monoesters (Span 20, 40, 60 and 80) and a sorbitan trioleate (Span 85) using a mechanical shaking technique without sonication. 5(6)-Carboxyfhxxescein (CFI was used as a model solute to investigate entrapment efficiency and release. For Span 80, cholesterol and dicetyl phosphate DCP) in the molar ratio 47.5: 47.5: 5.0, entrapment efficiency increased linearly with increasing concentration of lipid. Entrapment efficiency increased with increasing cholesterol content when vesicles were prepared by changing the molar ratio of non-ionic surfactant to cholesterol. Most efficient entrapment of CF occurred with Span 60

(HLB 4.7). Mean size of the un-sonicated niosomes showed a regular increase with increasing HLB from Span 85 (HLB 1.8) to Span 20 (HLB 8.6). The release rate of CF from vesicles depended on the surfactant used in the preparation of the vesicles.

3. Behrooz Nasseri et al., explained the effect of cholesterol and temperature on the elastic properties of niosomal membranes. The mechanical characteristics of non-ionic bilayer membranes composed of span 60, cholesterol and poly-24- oxyethylene cholesteryl were studied by measuring the modulus of surface elasticity (μ), a measure of membrane strength, as a function of cholesterol content and temperature. The modulus of surface elasticity increased slowly with increasing cholesterol concentration, with a sharp increase around 40 mol% cholesterol and displayed a maximum around 47.5 mol% cholesterol. Further cholesterol resulted in a decrease in μ. Generally, the interaction of cholesterol with the span 60 should increase the rigidity of the membrane. However, the latter effect may be due to the formation of *cholesterol clusters* at high cholesterol content where excess amounts of cholesterol cannot interact with the sorbitan monostearate, and deposits on the bilayers compromising their uniformity, strength and permeability.

4. Aranya Manosroi et al., studied the vesicles prepared with hydrated mixture of various non-ionic surfactants and cholesterol. The bilayer formation was characterized by X-cross formation under light polarization microscope. Membrane rigidity was measured by means of mobility of fluorescence probe as a function of temperatures. The stearyl chain (C18) non-ionic surfactant vesicles showed higher entrapment efficiency than the lauryl chain (C12) non-ionic surfactant vesicles. Cholesterol was used to complete the hydrophobic moiety of single alkyl chain non-ionic surfactants for vesicle formation.

5. Wei Hua et al., prepared highly stable innocuous niosome composed of only three components in Span 80/PEG 400/H2O system. The niosome properties are studied by some means of freeze fracture replication-transmission electron microscopy, negative staining-transmission electron microscopy, dynamic light scattering and differential scanning calorimetry. The obtained results indicate that the niosome can be stable for over one year. The niosome diameter is between 100 and 180 nm. The compositions of the system affect the preparation and properties of the niosome.

6. Prasun Bandyopadhyay et al., studied of the self-organization of nonionic surfactant span 60 in presence of fatty alcohol (stearyl, cetyl and lauryl) is presented. When ethanolic solution of the surfactant–fatty alcohol (1:1) mixture is added in water spontaneous large unilamellar vesicles (LUV) are formed. Vesicular suspension has been characterized by transmission electron microscopy, dynamic light scattering, confocal laser scanning microscopy, dye entrapment and release studies.

7. Ijeoma F. Uchegbu et al., summarized the achievements in the niosomes field. A number of groups worldwide have studied non-ionic surfactant vesicles (niosomes) with a view to evaluating their potential as drug carriers. Niosomes may be formed form a diverse array of amphiphiles. The self assembly of surfactants into niosomes is governed by the nature of the surfactant, the presence of membrane additives, the nature of the drug encapsulated and the actual method of preparation. The influence of formulation factors on niosome stability is also examined. Niosomes have been evaluated as immunological adjuvants, anti-cancer: anti-infective drug targeting agents, carriers of anti-inflammatory drugs, in diagnostic imaging, achieve transdermal and ophthalmic drug delivery.

8. Sandeep Kumar Sharma et al., prepared the niosomes with different molar ratios of surfactant and cholesterol using the drug fluconazole. Their morphological properties have been determined by scanning electron microscopy. Furthermore, the release profile, entrapment efficiency, size distribution and stability of these niosomes under various temperatures were studied.

9. Mahmoud Mokhtar Ahmed Ibrahim et al., formulated and evaluated proniosomal transdermal carrier systems for flurbiprofen. Proniosomes were prepared using various non-ionic surfactants, namely span 20, span 40, span 60 and span 80 without and with cholesterol at percentages ranging from 0% to 50%. The effect of surfactant type and cholesterol content on drug release was investigated. Drug release was tested by diffusion through cellophane membrane and rabbit skin; rabbit skin showed lower drug diffusion rates compared to cellophane membrane. Drug release studies showed the proniosomal composition controlled drug diffusion rates to be either faster or slower than the prepared flurbiprofen suspensions in HPMC gels or distilled water, respectively. Microscopic observations showed that either proniosomal solutions or gel formulations immediately converted to niosomal dispersions upon hydration.

10. Jia-You Fang et al., elucidated the skin permeation and partitioning of a fluorinated quinolone antibacterial agent, enoxacin, in liposomes and niosomes, after topical application. In vitro percutaneous absorption experiments were performed on nude mouse skin with Franz diffusion cells. A significant relationship between skin permeation and the cumulative amount of enoxacin in the skin was observed. Both permeation enhancer effect and direct vesicle fusion with stratum corneum may contribute to the permeation of enoxacin across skin.

Formulation with niosomes demonstrated a higher stability after 48 h incubation compared to liposomes. The inclusion of cholesterol improved the stability of enoxacin liposomes.

11. Ismail A. Attia et al., demonstrated the preparation of acyclovir niosomes in a trial to improve its poor and variable oral bioavailability. The niosomes were prepared by the conventional thin film hydration method. The % entrapment was found to be ~11%. The vesicles have an average size of 0.95 μm and a size range of 0.4 to 2.2 μm. Most of the niosomes have unilamellar spherical shape. The niosomal formulation exhibited significantly retarded release compared with free drug. The in vivo study revealed that the niosomal dispersion significantly improved the oral bioavailability (more than 2-fold increase) of acyclovir in rabbits. The niosomal dispersion showed significant increase in the MRT of acyclovir reflecting sustained release characteristics.

12. Manivannan Rangasamy et al., explained the acyclovir niosome preparation with different ratios of (1:1, 1:2, 1:3) cholesterol and Span 80 using hand shaking and ether injection process. The vesicles were in size range of 0.5-5 μm (hand shaking process) and 0.5-2.5 μm (ether injection process). The order of entrapment efficiency increases when Span 80 concentration was increased. *In vitro release* study indicates 76.64% for CHOL: Span 80 (1:1) formulation; takes an extended period of 1 day & 16hrs for release.

13. Pratap S. Jadon et al., developed griseofulvin niosomes to improve its poor and variable oral bioavailability. Niosomes were prepared by using span 20, span 40, and span 60. The formulations prepared by thin film method and ether injection method. The influence of different formulation variables such as surfactant type, surfactant concentration, and cholesterol concentration was optimized for size distribution and entrapment efficiency for

both methods. Higher entrapment efficiency obtained with span 60 niosomes prepared by thin film method. The niosomal formulation exhibited significantly retarded in vitro release as compared with free drug. The in vivo study revealed that the niosomal dispersion significantly improved the oral bioavailability, AUC of griseofulvin.

14. **Abdul Hasan Sathali A. et al.,** developed terbinafine Hcl niosomes to the fungal affected cells for targeted delivery. Niosomes formulated by thin film hydration method using different ratios of non ionic surfactant (Tween 20, 40, 60, and 80) and cholesterol with constant drug concentration. The formulations evaluated for its vesicle size (by AFM), entrapment efficiency (by dialysis method) *in vitro* release studies and antifungal activities. The formulation with surfactant cholesterol ratio 2:1 in each group of surfactant showed good entrapment. Niosomes tested for *in vitro* antifungal activity using the strain *Aspergillus niger* and compared with pure drug solution (as standard).. The best (Tween 40 niosomes) formulation incorporated into gel bases and evaluated.

15. Ghada Abdelbary et al., investigated the feasibility of using niosomes as carriers for the ophthalmic controlled delivery of gentamicin sulphate. Niosomes prepared using various surfactants (Tween 60, Tween 80 or Brij 35), cholesterol and a negative charge inducer DCP in different molar ratios by thin film hydration technique. The entrapment efficiency determined by centrifugation. Photomicroscopy, TEM and particle size analysis used to study the morphology and size of niosomes. Ocular irritancy test performed on albino rabbits, showed no sign of irritation for all tested niosomal formulations.

16. Kandasamy Ruckmani et al., evaluated the effect of process-related variables like hydration time, sonication time, rotation speed of evaporation flask, changes in osmotic shock, viscosity, the effects of charge-inducing agent, centrifugation on entrapment and release from zidovudine niosomes. Formulation of zidovudine niosomes was optimized by altering the proportions of Tween, Span and cholesterol. Non-sonicated niosomes were in the size range of 2-3.5 μm and sonicated niosomes had a mean diameter of 801 nm. Niosomes formulated with Tween 80 entrapped high amounts of drug and the addition of DCP enhanced drug release for a longer time (88.72% over 12 h). The mechanism of release was the Fickian type and obeyed first-order release kinetics.

17. Sankar V. et al., developed salbutamol sulphate niosomes to reduce the side effects during asthma treatment. Niosomes prepared by thin film hydration method. Highest % EE was found in span 60 formulation. The tissue distribution studies were carried out to determine the concentration of drug in lungs when given in niosomal form by using 4 groups of male mice. Pharmacokinetic studies were carried to predict the existence of drug in the systemic circulation by using 3 groups of New Zealand rabbits. It showed that enhanced tissue distribution and extended release of salbutamol sulphate when given in niosomal form.

18. Varaporn Buraphacheep Junyaprasert et al., investigated an influence of different types of membrane additives including negative charge (dicetylphosphate, DCP), positive charge (stearylamine, STR) and non-ionic molecule (cholesteryl poly-24-oxyethylene ether, SC24) on the physicochemical properties of drug-free and drug-loaded niosomes. Salicylic acid selected as a model drug. The niosomes were composed of 1:1 mole ratio of Span 60: cholesterol as vesicle forming agents. Addition of the membrane additives changed the zeta potential depending on the type of the additives. Transmission electron microscopy revealed

that niosomes had unilamellar structure. The particle sizes of all developed niosomes were between 217 to 360 nm. All niosomes showed no leakage of the salicylic acid after 3 months of storage indicating the good stability.

19. Tamizharasi S. et al., formulated gliclazide-loaded niosomes and evaluated for their *in vitro* as well as *in vivo* characteristic in an attempt to improve the oral bioavailability. Microscopic observation confirmed that all particles were uniform in size and shape. The entrapment efficiency determined dialysis method. The in vitro release studies exhibited a prolonged drug release over a period of 24 h. The positive values of zeta potential indicated that the gliclazide niosomes were stabilized by electrostatic repulsive forces. The niosomes showing maximum entrapment and suitable release rate were selected for in vivo evaluation.

20. **Vijay Prakash Pandey et al.,** demonstrated the ofloxacin niosomes formation, to overcome ofloxacin eye drop solution drawbacks (poor bioavailability) characterization. Niosomes prepared by lipid film hydration method using span 60 and cholesterol (various molar ratios); Characterized for entrapment efficiency, in-vitro drug release, surface charge, rheological character, physical stability, minimum inhibitory concentration, in-vivo drug release and ocular irritation studies. The span 60: cholesterol in molar ratio of 100:60 showed higher entrapment of drug and released 73.77 % at 10th hr and the availability of drug in the aqueous humor was 4.373µg/ml (Cmax), confirmed by HPLC method. The histopathology study also confirmed the safe use of niosomes.

21. **Shyamala Bhaskaran et al.,** investigated niosomes containing salbutamol sulphate using Span 60 as the surfactant, by employing different techniques namely, thin film

hydration, hand shaking, ether injection, lipid layer hydration and trans membrane pH gradient method. The drug entrapment efficiency varied from 62 % to 87 %. Transmembrane pH gradient method was found to be most satisfactory which released 78.4 % of drug in 24 h. Tissue distribution studies in albino rats and bio- availability studies in rabbits were carried out.

22. Anitha R. Desai et al., explained about the improvement in the efficacy, reduced toxicity and enhancement of therapeutic index of niosome carrying α-lipoic acid. Niosomes were prepared by reverse phase evaporation method using span and tween (20 and/or 60) and characterized for size reduction, entrapment efficiency, invitro drug release profile and stability under specific conditions. The diameter of niosome ranges from 1-3µm with spherical/ oval shape. Stability studies proved that optimum storage condition for niosomes was found to be 4ºC.

23. Raja Naresh R.A. et al., explained about diclofenac Na niosomes comprising Tween 85 & Tween 85-poloxamer F 108 mixture. Anti-inflammatory efficacy of these niosomes was compared with that of free diclofenac Na in adjuvant induced arthritic rats. It was found that the niosomal diclofenac Na formulations prepared by employing a 1:1 combination of Tween 85 & poloxamer F 108 elicits a better and consistent anti-inflammatory activity for more than 72 hrs after administration of a single dose.

24. Ajay B. Solanki et al., optimized the composition of niosomes containing aceclofenac for transdermal application, with a view to improve permeation of drug during an extended period of time. Niosomes were prepared by thin film hydration technique. A 3^2 factorial design was utilized to study the effect of the molar ratio of drug to lipid ($X1$) and

volume of hydration medium (*X*2) on percentage drug entrapment (PDE) and vesicle size. Selected batches of niosomes were incorporated in to carbopol gel matrix to prepare the niosomal gel formulations, which were evaluated for *in-vitro* release, skin permeation and *in vivo* studies. It was evident from the derived polynomial equations and constructed contour plot, a decrease in the level of *X*1 and an increase in the *X*2 lead to an increase in PDE and decrease in vesicle size. The polynomial equations and contour plot predicted the levels of independent variables *X*1 and *X*2 (0.19 and 0.46 respectively), for maximized response of PDE with constraints on vesicle size.

25. Anitha R. Desai et al., explained about the improvement in the efficacy, reduced toxicity and enhancement of therapeutic index of niosome carrying α-tocopherol. Niosomes were prepared by reverse phase evaporation method using span and tween (20 and/or 60) and characterized for size reduction, entrapment efficiency, invitro drug release profile and stability under specific conditions. The diameter of niosome ranges from 1-5µm with spherical/ round shape. Stability studies proved that optimum storage condition for niosomes was found to be 4ºC.

26. A. Manosroi et al., developed a novel elastic bilayer vesicle entrapped with NSAID, diclofenac diethylammonium (DCFD) for topical use. 18 formulations composing of DPPC or Tween 61 or Span 60 mixed with cholesterol and ethanol at 0–25% (v/v), by chloroform film method with sonication was developed. The elastic Tween 61 niosomes which gave no sedimentation, no layer separation, unchanged particle sizes (about 200 nm) were selected to entrap DCFD. Transdermal absorption through excised rat skin was performed by vertical

Franz diffusion cell at 32±2 ◦C for 6 h. The *in vivo* anti-inflammatory activity was evaluated by ethyl phenylpropiolate (EPP)-induced rat ear edema (n = 3).

27. Toshimitsu Yoshioka et al., described in which niosomes are dispersed in an aqueous phase which is then emulsified in an non-aqueous continuous phase. The resultant vesicle (niosome) –in-water-in-oil (v/w/o) system allows the delivery of vesicles in a non-aqueous vehicle. The non-ionic surfactants used to prepare the vesicles (niosomes) are also employed in the emulsification step to minimize surfactant redistribution. The invitro release rate of CF showed a decrease in the order free solution >vesicle suspension >w/o emulsion >v/w/o emulsion. The release rate of CF from the v/w/o system depends on the nature of the surfactants used.

28. Prabagar Balakrishnan et al., reported to improve the low skin penetration and bioavailability characteristics shown by topical vehicle for minoxidil. Niosomes were prepared with thin film hydration method using Brij, Span and cholesterol at various ratios. The prepared systems were characterized for entrapment efficiency, particle size, zeta potential and stability. Skin permeation studies were performed using static vertical diffusion Franz cells & hairless mouse skin. Higher entrapment efficiency was obtained with the niosomes prepared from Span 60, cholesterol at 1:1 molar ratio.

29. Vyas Jigar et al., investigated that the erythromycin (macrolide antibiotic) was entrapped into niosomes by thin film hydration method to avoid unwanted side effects, to enhance skin penetration as well as to improve skin retention. Various parameters were optimized by partial factorial design. The optimized niosomal formulation was incorporated into carbopol gel and extensively characterized for PDE & invitro release.

30. Anand kumar Y. et al.,, aimed at developing and optimizing niosomal formulation of aceclofenac in order to improve its bioavailability. In evaluation study the effect of the varying composition of non ionic surfactant and cholesterol on the properties such as encapsulation efficiency, particle size and drug release were studied. The drug release from the formulation was evaluated through dialysis membrane and extended over a period of 72 h in all formulations. The mechanism of dug release was governed by Peppas model.

31. Cosco D. et al., evaluated niosomes made up of bola, Span 80 & cholesterol (2:5:2 molar ratio) are proposed as suitable delivery systems for the administration of 5-fluorouracil (5-FU), an antitumoral compound largely used in the treatment of breast cancer. The bola-niosomes, after sonication procedure, showed mean sizes of ~200 nm and a loading capacity of ~40% with respect to the amount of 5-FU added during the preparation. Similar findings were achieved with PEG-coated bola-niosomes. 5-FU-loaded PEG-coated and uncoated bola niosomes were tested on MCF-7 and T47D cells. Both bola-niosome formulations provided an increase in the cytotoxic effect. Confocal laser scanning microscopy studies were carried out to evaluate both the extent and the time-dependent bola-niosome-cell interaction. In vivo experiments on MCF-7 xenograft tumor SCID mice models showed a more effective antitumoral activity of the PEGylated niosomal 5-FU at a concentration ten times lower (8 mg/kg) than that of the free solution of the drug (80 mg/kg) after a treatment of 30 days.

32. Naresh Ahuja et al., prepared the niosomes containing lansoprazole (antacid and anti ulcer agent) by using reverse phase evaporation method. Non-ionic surfactant Span 60 was use to prepare the formulations. Niosomes are characterized for its entrapment efficiency, size

range and invitro release of drug. For release study the phosphate buffered saline pH 8.6 was used and the samples were assayed by UV.

33. Udupa N., Chandraprakash K.S. et al., examined the methotrexate (MTX) niosomes by preparing it using the thin film hydration method with Tween 80, 60, 40, Span 60, 40 and 20. The MTX- entrapped niosomes were separated from the unentrapped by dialysis. Measurement of niosome size was made by using a microscope with a mean diameter 4.5µm. The entrapment efficiency has also been observed to be greater for Span 60 and least for Tween 80 containing niosomes. The reason may be attributable to the increased lipophilicity of Span 60. The order of entrapment efficiciency increase as the lippophilcity increased.

34. Jia You Fang et al., investigated the skin permeation of estradiol from various proniosomes gel formulations across excised rat skin in vitro. The encapsulation efficiency and size of niosomal vesicles formed from proniosomes upon hydration were also characterized. The % encapsulation of proniosomes with span surfactants showed a very high value of =100%. Proniosomes with span 40 and span 60 increased the permeation of estardiol across skin. Both penetration enhancer effect of non-ionic surfactant and vesicle-skin interaction may contribute to the mechanisms for proniosomes to enhance estradiol permeation.

35. Pavala Rani .N. et al., studied that niosomes are vesicles mainly consisting of non-ionic surfactants that encloses and encompasses the drug molecules. Niosomes of rifampicin and gatifloxacin were prepared by lipid hydration technique using rotary flash evaporator. The prepared rifampicin and gatifloxacin niosomes showed a vesicle size in the range of

100-300nm, the entrapment efficiency were 73% and 70% respectively. The *invitro* release study showed that 98.98% and 97.74% of release of rifampicin and gatifloxacin niosomes respectively. The bactericidal activities of the niosomal formulation were studied by BACTEC radiometric method using the resistant strains (RF 8554) and sensitive strains (H37Rv) of *Mycobacterium tuberculosis* which showed greater inhibition and reduced growth index.

36. **Sambhakar S et al., prepared** niosomes containing cefuroxime axetil was prepared by film formation method by Span 40, 60 and 80 to overcome the bioavailability problem (25%). It is characterised by SEM for particle size and morphology. Entrapment efficiency and release study was carried out by dialysis. In-vitro absorption study was carried out by everted sac method and also the stability study of niosomes in presence of bile salts was determined. The vesicle size was found to be less than 5 μm and its polydispersity index was very low. Entrapment efficiency was found as Span 60 > Span 40 > Span 80. The in-vitro-release study indicated the controlled release profile of niosomes.

CHAPTER-V

SCOPE OF WORK

Hypertension (or) high blood pressure is a medical condition where in the blood pressure is chronically elevated. Persistent hypertension is one of the risk factors for strokes, heart failure and arterial aneurysm, and is a leading cause of chronic renal failure.

Anti hypertensive drugs act by reducing the cardiac output and / or reducing the total peripheral resistance, without correcting the cause. Anti hypertensive drugs may ultimately reduce BP in humans by more than one mechanism. Further, the hemodynamic alterations produced by a single parenteral dose of a given drug may differ from the effects resulting from its prolonged oral administration [59].

Ramipril, a potent anti hypertensive drug is almost completely converted to its active metabolite ramiprilat (a dicarboxylic acid) by hydrolytic cleavage of the ester group in the liver, which has about 6 times the angiotension – converting enzyme inhibitor activity of ramipril. Ramipril categorized as a class IV / II drug according to biopharmaceutical classification system (BCS) because of its low solubility and poor permeability. Ramipril is a highly lipophilic log P (octanol / water) 3.22, poorly water soluble drug with absolute bioavailability of 28 – 30% of variable oral absorption. The poor oral bioavailability is due to the poor / low solubility and poor permeability **[52].**

Ramipril undergoes significant 'first pass metabolism'. The half life of ramipril and its metabolite is 2 and 18 hrs respectively.

Based on these, the purpose of this study is to prepare ramipril niosomes in a trial to improve its poor oral bioavailability and also to evaluate process related variables like sonication time, hydration time, osmotic shock, rotational speed of evaporator flask and the effects of charge inducing agent and release from niosomes. Ramipril niosomes were formulated by thin film

hydration method using different ratios of non-ionic surfactant (Span 60, 40, 20, 80 and Tween 60) and cholesterol with constant drug concentration.

Because niosomes, an alternative colloidal carrier (transport) system having ability to improve the solubility / permeability of lipophilic drugs and enhance drug absorption and bioavailability. Niosomes improve targeting and stability of drug.

Thus the objective of Niosomes as an alternative colloidal carrier system of ramipril was formulated by proper adjustment of process parameters to enhance ramipril entrapment and sustainability of release.

CHAPTER-VI

PLAN OF WORK

PART-I

1. Determination of max of Ramipril in phosphate buffered saline pH7.4.
2. Calibration curve for the drug in phosphate buffered saline pH7.4

PART-II

1. Formulation of ramipril loaded niosomes using different molar ratios of non ionic surfactant (Span 60) and constant molar ratio of cholesterol (30µmol) by thin film hydration method.

PART-III

Evaluation of Ramipril loaded niosomes

1. Determination of drug content
2. Determination of entrapment efficiency
3. In –vitro release studies of niosomes in phosphate buffered saline pH 7.4

PART-IV

The best molar ratio of nonionic surfactant, cholesterol was selected and used for the preparations of formulations containing different nonionic surfactants (Span 20. Span 40, Span 80, Tween 60).

PART-V

1. Determination of drug content
2. Determination of entrapment efficiency
3. In –vitro release studies of niosomes in phosphate buffered saline pH 7.4

PART-VI

The best formulation was selected and used for various parameters optimization.

1. Viscosity determination
2. Effect of sonication time
3. Effect of hydration time
4. Effect of osmotic shock
5. Effect of rotational speed of evaporator flask
6. Effect of charge inducing agents (Stearylamine, Dicetyl phosphate).

PART-VII

Invitro release studies of ramipril niosomes containing STR(5µmol), DCP(10µmol) and ramipril drug solution phosphate buffered saline pH 7.4.

PART-VIII

1. Measurement of particle size of ramipril loaded niosomes.
2. Morphological studies of niosomes using scanning electron microscopy
3. Zeta potential measurement of the niosomes using Malvern Zeta Sizer

PART-IX

1. Differential scanning calorimetry studies of selected formulations to determine the status of the drug, nonionic surfactant and cholesterol.
2. Fourier Transform Infra Red Spectroscopy (FT-IR) studies to determine interaction between the drug, nonionic surfactant and cholesterol.

PART-X

Stability studies of niosomes at refrigerated and room temperature.

CHAPTER-VII

MATERIALS AND EQUIPMENTS

MATERIALS USED

1. Drug- Ramipril	- Madras Pharmaceuticals.
2. Cholesterol	- S.D.Fine chem. Ltd
3. Sorbiton monolaurate	- Loba chemie
4. Sorbiton monopalmitate	- Loba chemie
5. Sorbiton monostearate	- Loba chemie
6. Sorbiton monooleate	- Loba chemie
7. Polyoxyethylene-20 sorbitan monostearate	-S.D.fine Chem Ltd.
8. Chloroform	- Rankem
9. Methanol	- Rankem
10. n-propanol	- Nice Chemicals
11. Sodium chloride	- Central drug house
12. Potassium dihydrogen ortho phosphate	- Nice chemicals
13. Disodium hydrogen ortho phosphate	- Qualigens
14. Dialysis memebrane 50 – LA 387	- Himedia

EQUIPMENTS USED

1. Rotary Flash Evaporator	- Super fit rotary flash evaporator
2. Ultra Sonicator	- Vibronic's Ultrasonic processor
3. Electronic Balance	- A&D Company, Japan
4. Magnetic Stirrer	- MC Dalal & co
5. UV Visible Spectrophotometer	- UV Pharma Spec 1700, Shimadzu
6. Cooling Centrifuge Apparatus	- Eppendorf Centrifuge 5417R
7. Particle size analyzer	- Blue wave
8. Malvern zeta sizer	- Malvern zeta sizer Nano ES-90
9. Scanning electron microscope	- Hitachi S-3400
10. FT-IR Spectrophotometer	- Shimadzu
11. Differential Scanning Calorimeter	- Perkin Elmer STA 6000 Thermal Analyzer
12. Refrigerator	- Kelvinator
13. Environmental chamber	- Inlab equipments(Madras) Pvt. Ltd

CHAPTER-VIII

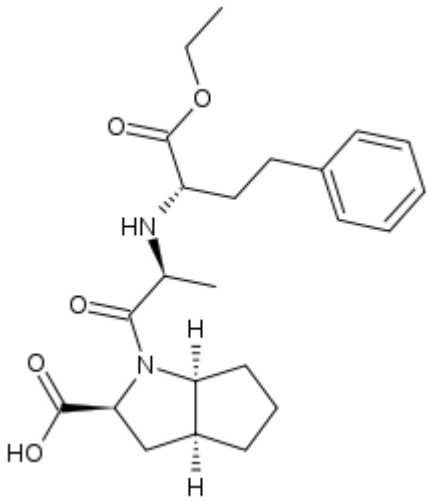
DRUG PROFILE

RAMIPRIL [36, 37, 38, 39, 40, 41, 42, 43, 44, 68, 70, 71].

Synonym:

Ramiprilum

Structure



Systematic IUPAC name

(2S, 3aS, 6aS) -1- [(2S) -2-[[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino]propanoyl]-3,3a,4,5,6,6a- hexahydro-2H-cyclopenta[d]pyrrole-2-carboxylic acid.

Chemical Formula

$C_{23}H_{32}N_2O_5$

Description

Nature	:	White crystalline powder
Solubility	:	Freely soluble in methanol,
		Sparingly soluble in water.
Melting point	:	109°C
Molecular weight	:	416.511gm/mol
Log P (octanol/water)	:	3.32(Ramipril)
Octanol/water partition coefficient	:	0.06 (Ramipril)
		0.006 (Ramiprilat)
pKa	:	3.1, 5.6 (Ramipril)
		1.55, 3.44 (Ramiprilat)

Category

- Angiotensin-converting Enzyme Inhibitors.
- Antihypertensive Agents.

Identification

UV light absorption at 207 nm.

Pharmacodynamic Properties

Ramipril an angiotensin-converting enzyme inhibitor (ACE inhibitor). Ramipril an inactive prodrug is converted to active metabolite ramiprilat in liver. Ramiprilat the active metabolite competes with angiotensin converting enzyme blocking the conversion of angiotensin I to angiotensin II. It is a vasoconstrictor and a negative feedback mediator for renin activity. Lower concentrations result in decrease in blood pressure and an increase in

plasma renin. Ramiprilat may also act on kininase II, an enzyme identical to ACE that degrades vasodilator bradykinin.

Pharmacokinetic Properties

Absorption

- ✶ Extent of absorption in gastrointestinal tract is atleast 50 % to 60 %.
- ✶ T max is 1 hour for parent compound, 2 to 4 hour for metabolite.
- ✶ Plasma half life 2 to 4 hours.

Metabolism

- ✶ Ramipril is converted to active metabolite Ramiprilat in liver by the enzyme
- ✶ Ramiprilat has 6 times greater ACE inhibition activity than the parent compound.

Excretion

- ✶ 60 % of parent compound and metabolites are excreted in urine.
- ✶ 40 % of parent compound and metabolites are excreted in faeces.
- ✶ Less than 2 % of unchanged drug excreted in urine.

Pharmacokinetic Characters of Ramipril

➢ Oral Bioavailability	:	28 % (Ramipril) 44% (Ramiprilat)
➢ Excretion	:	Renal (60 %), Fecal (40 %).
➢ Plasma protein binding	:	73 % (Ramipril) 56 % (Ramiprilat)

Therapeutic Indications

- ✶ Control of hypertension;
- ✶ Treatment of congestive heart failure;
- ✶ Myocardial Infarction;
- ✶ To prevent stroke, cardiovascular death;
- ✶ Diabetic nephropathy with microalbuminuria.

Dose

5 to 10 mg per day.

Storage

Protected from light.

Side Effects

- ✶ Postural Hypotension
- ✶ Hyperkalemia
- ✶ Dry cough
- ✶ Angio edema
- ✶ Neutropenia
- ✶ Agranulocytosis
- ✶ Anaphylactic reactions
- ✶ Nausea
- ✶ Vomiting
- ✶ Dizziness
- ✶ Change in amount of urine
- ✶ Yellowing of eyes or skin, dark urine

Drug Interactions

- ✪ Hyperkalemia with potassium sparing diuretics and potassium supplements.
- ✪ Antacids reduce bioavailability of Ramipril.
- ✪ Indomethacin (and other NSAIDS) attenuate the hyoptensive action.
- ✪ Tizanidine increases the risk of hypotension with the ACE inhibitor.

Special Precautions

- ✪ Do not take potassium supplements without seeking medical advice
- ✪ Do not take during pregnancy.

Contra-indications

- ✶ Reno vascular disease.
- ✶ Severe renal impairment.
- ✶ History of angio edema.
- ✶ During Pregnancy.
- ✶ Hypotension.
- ✶ High-dose diuretic therapy.
- ✶ Salt and water-depleted states.
- ✶ Use of potassium-sparing diuretics.

International Brand Names

- Acovil (Spain)
- Cardace (India, Indonesia)
- Delix (Germany, Turkey)
- Hyperil (ID)
- Hypren, (Austria)
- Lostapres (Argentina)
- Ramace (Australia)
- Tritace (Argentina, Australia)
- Unipril (Italy)
- Vesdil, Promed (Germany).

CHAPTER-IX

EXCIPIENTS PROFILE

SORBITAN MONOLAURATE [45]

SYNONYM

Arlacel 20; Crill 1; Liposorb L; Montane 20; Sorbitan laurate; Span 20.

CHEMICAL NAME

Sorbitan mono dodecanoate.

EMPIRICAL FORMULA

C_{18} H_{34} O_6

MOLECULAR WEIGHT

346

DESCRIPTION

Yellow viscous liquid.

METHOD OF MANUFACTURE

Sorbitol is dehydrated to form a hexitan (1,4-sorbitan), which is then esterified with the desired fatty acid.

PROPERTIES

Acid value	– ≤7
Hydroxyl value	– 159-169
Saponification value	– 159-169
Density	1.01g/cm^3
HLB Value	– 8.6

FUNCTIONAL CATEGORY

- Emulsifying agent
- Nonionic surfactant
- Solubilizing agent
- Wetting agent
- Dispersing / suspending agent.

STABILITY

- Gradual soap formation occurs with strong acids or bases
- Stable in weak acids or bases.

STORAGE

It should be stored in a well-closed container in a cool, dry place.

SAFETY

Daily intake according to the WHO limit is about 25mg/Kg body weight .

HANDLING PRECAUTIONS

Eye protection and Gloves are recommended.

SORBITAN MONOPALMITATE [45]

SYNONYM

Arbunol S-40; Alracel 40; Crill 2; Liposorb P; Montane 40; Sorbitan palmitate; Span 40.

CHEMICAL NAME

Sorbitan mono hexadecanoate.

EMPIRICAL FORMULA

C_{22} H_{42} O_6

MOLECULAR WEIGHT

403

DESCRIPTION

Cream solid.

METHOD OF MANUFACTURE

Sorbitol is dehydrated to form a hexitan (1,4-sorbitan), which is then esterified with the desired fatty acid.

PROPERTIES

Acid value – 3-7

Hydroxyl value – 270-303

Saponification value – 142-152

Melting point 43-48ºC

Density (g/cm^3) – 1.0g/cm^3

HLB Value – 6.7

FUNCTIONAL CATEGORY

- Emulsifying agent
- Nonionic surfactant
- Solubilizing agent
- Wetting agent
- Dispersing / suspending agent.

STABILITY

- Gradual soap formation occurs with strong acids or bases
- Stable in weak acids or bases.

STORAGE

It should be stored in a well-closed container in a cool, dry place.

SAFETY

Daily intake according to the WHO limit is about 25mg/Kg body weight.

HANDLING PRECAUTIONS

Eye protection and Gloves are recommended.

SORBITAN MONOSTEARATE [45]

SYNONYM

Arbunol S-60; Alracel 60; Crill 3; Liposorb S-K; Montane 60; Sorbitan stearate; Span 60; Tego SMS.

CHEMICAL NAME

Sorbitan mono-octadecanoate.

EMPIRICAL FORMULA

C_{24} H_{46} O_6

MOLECULAR WEIGHT

431

DESCRIPTION

Cream solid.

METHOD OF MANUFACTURE

Sorbitol is dehydrated to form a hexitan (1,4-sorbitan), which is then esterified with the desired fatty acid.

PROPERTIES

Acid value	– 5-10
Hydroxyl value	– 235-260
Saponification value	– 147-157
HLB Value	– 4.7
Melting point	□53-57ºC

FUNCTIONAL CATEGORY

- Emulsifying agent
- Nonionic surfactant
- Solubilizing agent
- Wetting agent
- Dispersing / suspending agent.

STABILITY

- Gradual soap formation occurs with strong acids or bases
- Stable in weak acids or bases.

STORAGE

It should be stored in a well-closed container in a cool, dry place.

SAFETY

Daily intake according to the WHO limit is about 25mg/Kg body weight .

HANDLING PRECAUTIONS

Eye protection and Gloves are recommended.

SORBITAN MONOOLEATE [45]

SYNONYM

Albunol S-80; Arlacel 80; Crill 4; Liposorb O; Montane 80; Sorbitan oleate; Span 80; Tego SMO.

CHEMICAL NAME

(Z)-Sorbitan mono-9-octadecanoate.

EMPIRICAL FORMULA

C_{24} H_{44} O_6

MOLECULAR WEIGHT

429

DESCRIPTION

Yellow viscous liquid.

METHOD OF MANUFACTURE

Sorbitol is dehydrated to form a hexitan (1,4-sorbitan), which is then esterified with the desired fatty acid.

PROPERTIES

Acid value	– ≤8
Hydroxyl value	– 193-209
Saponification value	– 149-160
Density	□1.01g/cm^3
HLB Value	– 4.3

FUNCTIONAL CATEGORY

- Emulsifying agent
- Nonionic surfactant
- Solubilizing agent
- Wetting agent
- Dispersing / suspending agent.

STABILITY

- Gradual soap formation occurs with strong acids or bases
- Stable in weak acids or bases.

STORAGE

It should be stored in a well-closed container in a cool, dry place.

SAFETY

Daily intake according to the WHO limit is about 25mg/Kg body weight.

HANDLING PRECAUTIONS

Eye protection and Gloves are recommended.

POLYSORBATE 60 [45]

SYNONYM

Atlas 70K; Crillet 3; Glycosporse S-20; Liposorb S-20; Polyoxyethylene 20 stearate; Sorbitan monooctadecanoate; Tween 60, Tween 60K, Tween 60 VS.

CHEMICAL NAME

Polyoxyehylene 20 sorbitan monostearate.

EMPIRICAL FORMULA

C_{64} H_{126} O_{26}

MOLECULAR WEIGHT

1312

DESCRIPTION

Yellow oily liquid.

METHOD OF MANUFACTURE

Polysorbates are prepared from sorbitol in a three-step process. Water is initially removed from the sorbitol to form a sorbitan (a cyclic sorbital anhydride). The sorbitan is then partially esterified with a fattyacid such as stearic acid to yield a hexiton ester. Finally, ethylene oxide is chemically added in the presence of a catalyst to yield the polysorbate.

PROPERTIES

Acid value	– 2.0
Hydroxyl value	– 81 – 96
Saponification value	– 45 - 55
HLB Value	– 14.9
Solubility	– Soluble in ethanol and water.
	Insoluble in mineral oil and vegetable oil.

FUNCTIONAL CATEGORY

- Emulsifying agent
- Nonionic surfactant
- Solubilizing agent
- Wetting agent
- Dispersing / suspending agent.

STABILITY

- Stable to electrolytes and weak acids and bases.
- Gradual saponification occurs with strong acids and bases.
- It is hygroscopic and shoud be examined for water content prior to use and dried if necessary.
- Also, in common with other polyoxyethylene surfactants, prolonged storage can lead to the formation of peroxides.

STORAGE

It should be stored in a well-closed container protected from light, in a cool, dry place.

SAFETY

Daily intake according to the WHO limit is about 25mg/Kg body weight and moderately toxic by IV route.

REGULATORY STATUS

GRAS listed; Accepted as food additive; Included in the FDA Inactive Ingredients Guide (IM, IV, oral, rectal, topical and vaginal preparations).

HANDLING PRECAUTIONS

Eye protection and Gloves are recommended.

CHOLESTEROL[45]

SYNONYM

Cholesterolum; Cholesterin.

CHEMICAL NAME

Cholest -5- en-3β -ol.

EMPIRICAL FORMULA

$C_{27}H_{46}O$

MOLECULAR WEIGHT

386.67

FUNCTIONAL CATEGORY

- Emulsifying agent
- Emollient

DESCRIPTION

- Cholesterol occurs as white or faintly yellow, almost odourless, pearly leaflets, needles, powder or granules.
- On prolonged exposure to light and air, it acquires a yellow to tan color.

PROPERTIES

Boiling Point –360 ºC

Density –1.052g/cm^3 for anhydrous form

Melting Point –147-150ºC

Solubility _Soluble in acetone and vegetable oils.

Practically insoluble in water

Soluble in chloroform: methanol mixture.

STABILITY AND STORAGE CONDITIONS

It is stable, and should be stored in a well-closed container and protected from light.

METHOD OF MANUFACTURE

The commercial material is normally obtained from the spinal cord of cattle by extraction with petroleum ethers, but it may also be obtained from wool fat. Purification is normally accomplished by repeated bromination. Cholesterol may also be produced by entirely synthetic means.

Cholesterol produced from animal organs will always contain cholestanol and other saturated sterols.

SAFETY

It is generally regarded as an essentially non-toxic and non-irritant material at the levels employed as an excipient.

HANDLING PRECAUTIONS

Rubber or plastic gloves, eye protection and a respirator are recommended.

REGULATORY STATUS

Induced in the FDA inactive ingredients.

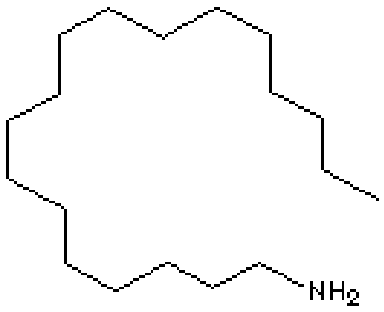
STEARYLAMINE [46, 47]

SYNONYM

Octadecylamine; Stearamine; n-Stearylamine;; 1- Octadecylamine;

n- Octadecylamine; Monooctadecylamine; Oktadecylamin.

STRUCTURE



CHEMICAL NAME

1-Aminooctadecane; 1-Octadecanamine

EMPIRICAL FORMULA

$CH_3 (CH_2)_{17}NH_2$

MOLECULAR WEIGHT

269.52g/mol

FUNCTIONAL CATEGORY

Cationic surfactants (disinfectants, fungicides, germicide, leveling agents, hair rinse bases, wood preservatives, textile softeners, dyeing auxiliaries, ore flotation. pigment grinding aids. anticaking agents)

- Amphoteric surfactants and Amine oxides (antistatic agent, textile scouring agent, ingredient for low irritation shampoo, liquid detergent, foam booster, oil recovery agent)

- Corrosion inhibitors and asphalt emulsifier

- Dispersants, lubricants, water treatment agents.

DESCRIPTION

White to off-white solid.

PROPERTIES

Physical state	:	White to off-white solid.
Melting point	:	47 - 53 °C
Boiling point	:	232 °C
Solubility in water	:	practically insoluble
Solvent solubility	:	soluble in methanol and chloroform mixture
Amine content	:	190-213 mg/g
Iodine value	:	3.0 g/100g

STABILITY AND STORAGE CONDITIONS

It is stable under ordinary conditions, and should be stored in a well-closed container and protected from light.

SAFETY

It is generally regarded as an essentially non-toxic and non-irritant material at the levels employed as an excipients.

HANDLING PRECAUTIONS

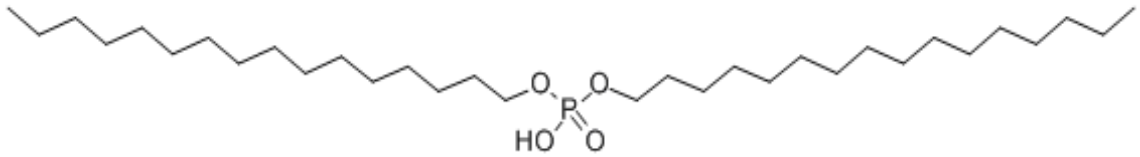
Keep away from heat. Keep away from sources of ignition. Empty containers pose a fire risk.

DICETYL PHOSPHATE [48]

SYNONYMS

Phosphoric acid dihexadecyl ester; Dihexadecyl hydrogen Phosphate; Dihexadecyl phosphate.

STRUCTURE



CHEMICAL NAME

1-Hexadecanol,hydrogenphosphate;bis(hexadecyl)phosphate; dicetylhydrogenphosphate; di-n-hexadecylphosphate.

EMPIRICAL FORMULA

$C_{32}H_{67}O_4P$

MOLECULAR WEIGHT

546.85g/mol

DESCRIPTION

anionic

FUNCTIONAL CATEGORY:

- Emulsifying agent.

- Non ionic Surfactant.
- Solubilizing agent.
- Wetting agent.

PROPERTIES:

Melting point: 74-75 °C (lit.)

STORAGE

It should be stored in a well-closed container at -20º C

CHAPTER- X

EXPERIMENTAL PROTOCOL

PREPARATION OF CALIBRATION MEDIUM [49]

Phosphate buffered saline pH 7.4

Dissolve 2.38 g of disodium hydrogen phosphate, 0.19 g of potassium dihydrogen phosphate and 8.0 g of sodium chloride in sufficient quantity of distilled water and the volume made up to 1000ml.

STANDARD CURVE FOR RAMIPRIL.

100mg of ramipril is accurately weighed and dissolved in a small quantity of methanol and made up to 100ml with the buffer phosphate buffered saline pH 7.4. From this primary solution 10ml is pipetted out and made up to 100ml with phosphate buffered saline pH 7.4. From this secondary solution aliquots are taken to produce 5, 10, 15 20, 25, 30, 35, 40, 45, 50µg/ml.

The absorbance of the resulting solution is measured at 207nm in the UV-Visible Spectrophotometer (Shimadzu UV-1700 Pharma spec Japan) using phosphate buffered saline pH 7.4 as blank. The standard curve is plotted by taking concentration in X-axis and Absorbance in Y-axis

PREPARATION OF RAMIPRIL LOADED NIOSOMES:

Thin Film Hydration Method [50, 51, 52, 66, 67, 69, 72]

Different ratios of surfactant and cholesterol are used to prepare niosomes as shown in table no: 8 with the concentration of the drug being the same.

The niosome formulations are prepared by **thin film hydration technique**. The weighed amount of cholesterol, non-ionic surfactant (cholesterol: non-ionic surfactant in micromoles) dissolved in 5ml of solvent mixture (Chloroform : Methanol 2:1 ratio). It is then transferred to a 100ml round bottom flask. A thin film is formed under reduced pressure in a rotary flash evaporator rotated at 100rpm at 55ºC.

The organic solvent is evaporated to form a dry film on the walls of the flask. An appropriate amount of ramipril is dissolved in phosphate buffered saline pH 7.4 and this is added slowly to the round bottom flask having thin film of surfactant and cholesterol and vortexed continuously for a period of 30 minutes at 55ºC, until a good dispersion of the mixture is obtained. The niosomal dispersion is collected and measured. The niosomal suspension is left to mature overnight at 4ºC.

The empty niosomes also prepared by the same method without the drug.

Drug Content Analysis [53, 54]

The amount of drug in the formulation is determined after lysing the niosomes using 50% n- propanol.

Niosomes preparation equivalent to 500µg of ramipril (1ml) is pipetted out in 100ml standard flask. To this sufficient quantity of 50% n- propanol is added and shaken well for the complete lysis of the vesicles. The volume is made up to 100 ml with the buffer phosphate buffered saline pH 7.4.

The absorbance is measured at 207nm in the UV-Visible Spectrophotometer (Shimadzu UV-1700 Pharma spec Japan) using empty niosomes as blank.

The drug content is calculated from the standard curve, by using the following formula,

$$\text{Drug content} = \frac{\text{Sample Absorbance}}{\text{Standard Absorbance}} \times 100.$$

Estimation of Entrapment Efficiency [55, 56]

Ramipril niosome preparations (1 ml) are centrifuged at 14,000 rpm for 90 minutes at 4ºC using a refrigerated centrifuge (Eppendorf, 5417R, Germany) in order to separate niosomes from unentrapped drug. The free drug concentration in supernatant layer after centrifugation are determined at 207 nm using UV-Visible Spectrophotometer (Shimadzu UV-1700 Pharma spec Japan). The percentage of drug entrapment in niosomes is calculated using the following formula

$$\% \text{ drug entrapment} = \frac{\text{(Total drug – Drug in supernatant)}}{\text{Total drug}} \times 100.$$

Invitro Release Study [50, 51, 52, 53]

Invitro release pattern of niosomes suspension is carried out by dialysis bag (Himedia dialysis membrane (mw 12,000) method. The niosomal preparation of ramipril is placed in a dialysis bag with an effective length of 5 cm which acts as a donor compartment. Dialysis bag is placed in a beaker containing 250 ml of buffer phosphate buffered saline pH 7.4, which acts as receptor compartment. The temperature of receptor medium maintained at 37±1ºC and the medium is agitated at 50 rpm speed using magnetic stirrer. Aliquots of 5 ml samples are collected at predetermined time and replenished immediately with the same volume of fresh buffer phosphate buffered saline pH 7.4. The sink condition is maintained throughout the experiment. The collected samples are analyzed spectrophotometrically at 207 nm using UV-

Visible Spectrophotometer (Shimadzu UV-1700 Pharma spec Japan). Each study is performed in triplicate.

The invitro release studies are also carried out for the pure drug by same method.

OPTIMIZATION OF PROCESS-RELATED VARIABLES

Determination of Viscosity [54]

Viscosity of the formulation is determined using an Ostwald viscometer at room temperature.

Effect of Sonication Time [54]

The niosomal formulation containing Span 60 1:1 (30µmol) is subjected to ultrasonic vibration using Vibronic's Ultrasonic Processor. To study the effect of sonication time, the formulation is subjected to sonication for various time intervals (like 1min, 2mins, 3mins, 4mins and 5mins). Particle size and entrapment efficiency of the formulation are measured.

Particle size analysis [57, 58]

The mean diameter of niosome in the dispersion is determined by Photon Correlation Spectroscopy (PCS) at a fixed angle of 90° at 25°C. Particle size is determined by using blue wave (microtac) using disposable sizing cuvette.

Effect of Hydration Time [54]

The niosomal formulation containing Span 60 1:1 (30µmol) is hydrated with 5.5 ml of phosphate buffered saline pH 7.4, for 30 minutes, 45minutes, 60minutes, 75minutes and 90minutes. The entrapment efficiency of the formulations is calculated by ultracentrifugation method.

Effect of Osmotic Shock [54, 55]

The effect of osmotic shock on niosomal formulations is investigated by monitoring the change in vesicle diameter after incubation of niosome suspensions in media of different tonicity 1.6% NaCl (hypertonic), 0.9% NaCl (normal) and 0.5% NaCl (hypotonic). Suspensions are incubated in these media for 3 hours and the change in vesicle size is measured by optical microscopy with a calibrated eyepiece micrometer.

Effect of Rotational Speed of Evaporator Flask [54]

The thickness and uniformity of the film depended upon the rotational speed of the evaporator flask. The niosomal formulation containing Span 60 1:1 (30µmol) is prepared with the speed of 75 rpm, 100 rpm, and 150 rpm of evaporator flask. The appearance of the formulations is checked by visual observation. The entrapment efficiency of the formulations is calculated by ultracentrifugation method.

Effect of charge inducing agents [56]

The effect of charge inducing agents on niosomal formulations is investigated by measuring the zeta potential measurement using Malvern Zeta Sizer (Malvern Zeta Sizer Nano ES-90, England). The optimized parameters used to prepare the selected formulation (F1) to study the effect of charge inducing agents. The niosomal formulations containing Span 60 1:1 (30µmol) are prepared with positive charge inducing agent (Stearylamine- STR, F11-5 µmol), negative charge inducing agent (Dicetyl Phosphate- DCP F12-5µmol, F13-10µmol and F14-5µmol) respectively.

The release studies carried out using dialysis bag method and the zeta potential of the prepared niosome containing DCP (F12) is measured at Malvern Zeta Sizer using disposable sizing cuvette.

Kinetics of drug release: [50, 54]

To understand the pharmacokinetics and mechanism of drug release, the result of *in-vitro* drug release study of niosomes were fitted with various pharmacokinetic equations like zero order (cumulative % release vs time), first order (log % drug remaining vs time), Higuchi's model (cumulative % drug release vs. square root of time), and the Korsmeyer-Peppas (log cumulative % drug release vs log time) and Hixson- Crowel models (cubic root of drug remaining vs time). The r^2 and k values were calculated for the linear curve obtained by regression analysis of the above plots.

Scanning electron microscopy [59]

Average particle size and surface morphology of niosomes are evaluated using scanning electron microscopy. The sample was spread on an aluminium stub and allowed to dry at room temperature. The dried sample then was sputter coated with gold for 40 seconds using Hitachi Ion-Sputter E-1010. The images were captured with the Hitachi S-3400 Scanning Electron Microscope.

FT-IR Studies [58, 73]

The possibility of drug-excipients (cholesterol, nonionic surfactants) interactions are further investigated by FT-IR spectrum study. The FT-IR spectrum of pure drug and combination of drug with excipient are obtained by using Shimadzu FT-IR Spectrophotometer. The scanning range is 450-4000 cm^{-1} and the resolution is $4cm^{-1}$. Samples are prepared in KBr pellets.

Differential Scanning Calorimetry [60]

Differential Scanning Calorimetry is performed using Perkin Elmer STA 6000 Thermal Analyzer. The instrument is calibrated with indium standard. Accurately weighed (it varies from 3mg-25mg) samples are placed in an open type ceramic sample pans. Thermo grams are obtained by heating the sample at a constant heating rate of 8ºC/minute. A dry purge of Argon gas (60ml/min) is used for all runs. Samples heated from 37ºC-400ºC.

Stability studies [50, 61]

The best formulation of ramipril loaded niosomes is subjected to stability studies. The formulation is stored in two different temperatures, 4 ± 2^0C, 25 ± 2^0 C/60% RH±5% RH in an environmental chamber [Inlab equipments (Madras) Pvt. Ltd] for the period of one month. The drug content, entrapment efficiency of the formulation is estimated every week.

CHAPTER- XI

RESULTS AND DISCUSSION

STANDARD CURVE OF RAMIPRIL [49]

The max of ramipril was determined by scanning the 10µg/ml of the drug solution in phosphate buffered saline (PBS) pH 7.4. It showed the λ max of 207nm in phosphate buffered saline pH 7.4.

Linear correlation coefficient was obtained for calibration of ramipril in phosphate buffered saline. Ramipril obeys the beer's law within the concentration range of 5 to 50 µg/ml. Calibration plot of ramipril in phosphate buffered saline pH 7.4 was shown in figure:10, 11 & table no: 6. The λ max of ramipril is showed in UV graph.

PREFORMULATION STUDIES:

Niosomal formulations were prepared by taking equimolar concentration of (1:1 ratio) non-ionic surfactant (Span 60) and cholesterol. The concentrations of the formulations are kept at 20, 30, 40, 50, 60 and 70 µmol with a fixed concentration of ramipril. The entrapment efficiencies of the formulations were determined by ultracentrifugation process and shown in table no: 7.The ranges of entrapment efficiency of six niosomal formulations were observed about 25.94% to 35.30%. From these formulations, it was observed that the formulation containing 30µmol concentration of non-ionic surfactant and cholesterol had higher entrapment efficiency. Therefore concentration of cholesterol was fixed a constant value (30µmol) for all formulations.

Effect of cholesterol concentration on entrapment efficiency: [62, 63]

Cholesterol is one of the common and essential additives in niosome formulations. The influence of added cholesterol within the lipid composition was observed by determining the percentage entrapment efficiency for the formulations containing molar concentrations from 20 µmol to 70 µmol table no: 7.

The order of entrapment efficiency as follows;

35.3% (30µmol) > 34.11% (40µmol) > 32.28% (20µmol) > 30.62% (50µmol) > 28.33% (60µmol) > 25.94% (70µmol)

Inclusion of cholesterol increases the viscosity of the formulation indicating more rigidity of the bilayer membrane. Cholesterol has the ability to cement the leaking space in the bilayer membranes. Cholesterol was found to have little effect on the ramipril entrapment. However, a significant increase in entrapment efficiency of ramipril was obtained when 30 µmol of cholesterol was incorporated into niosomes prepared from Span 60 followed by a decrease in entrapment efficiency of the drug upon further increase in cholesterol content, it was due to when the cholesterol content increases beyond a certain level, it starts disrupting the regular bilayer structure thereby decreases the drug entrapment.

It was suggested that, the improvements in drug loading observed when the concentration of cholesterol 30µmol. And the major reduction percentage entrapment efficiency when cholesterol content was further increased may be due to two conflicting factors:

(1) With increasing cholesterol, the bilayer hydrophobicity and stability increased and permeability decreased which leads to efficiently trapping the hydrophobic drug into bilayers as vesicles formed.

(2) In contrast, higher amounts of cholesterol may compete with the drug for packing space within the bilayer, hence excluding the drug as the amphiphiles assemble into vesicles.

Another study suggested that decreasing the entrapment efficiency with increasing cholesterol ratio above a certain limit may be due to the fact that increasing cholesterol beyond a certain concentration can disrupt the regular linear structure of vesicular membranes. Moreover Span 60 have higher phase transition temperature being solid at room temperature. Below transition temperature cholesterol made the membrane less ordered and increasing cholesterol has been found to increase membrane fluidity to the extent where the phase transition is abolished.

PREPARATION OF RAMIPRIL LOADED NIOSOMES:

Ramipril loaded niosomes was prepared by using increasing molar ratios of non ionic surfactant (Span 60) and constant molar ratio of cholesterol (30µmol) by thin film hydration method. From those formulations 1:1 ratio (30µmol) selected due to their higher entrapment efficiency and used for the ramipril loaded niosomes containing Span 20, Span 40, Span 80 and Tween 60. [66, 67] The formulation details are shown in table no: 8.

ENTRAPMENT EFFICIENCY

In all the formulations, the concentration of cholesterol and surfactant on entrapment efficiency is considerably significant. The range of entrapment efficiency of the niosomal formulations were observed about 22.05% to 35.05 % and are shown in table no: 9 & figure 12.

Higher entrapment efficiency was obtained with Span 60 niosomes (1:1 ratio 30µmol) may be due to surfactant chemical structure (Span series) and having highest phase transition temperature. [62, 63, 64]

Effect of non-ionic surfactants on entrapment efficiency:

The entrapment efficiency was 35.05 % for F1 formulation whereas it was 30.76 %, 29.16 %, 28.06 %, 27.57 % and 25.28 % for formulations F2, F3, F4, F5 and F6 respectively. This explains that **equimolar mixture has higher entrapment efficiency** compared with increasing concentration of surfactant while cholesterol content was maintained at a constant value.

The order of entrapment efficiency as follows;

35.05 % (F1 1:1) > 30.76 % (F2 1:2) > 29.16 % (F3 1:3) > 28.06 % (F4 1:4) > 27.57 % (F5 1:5) > 25.28 % (F6 1:6).

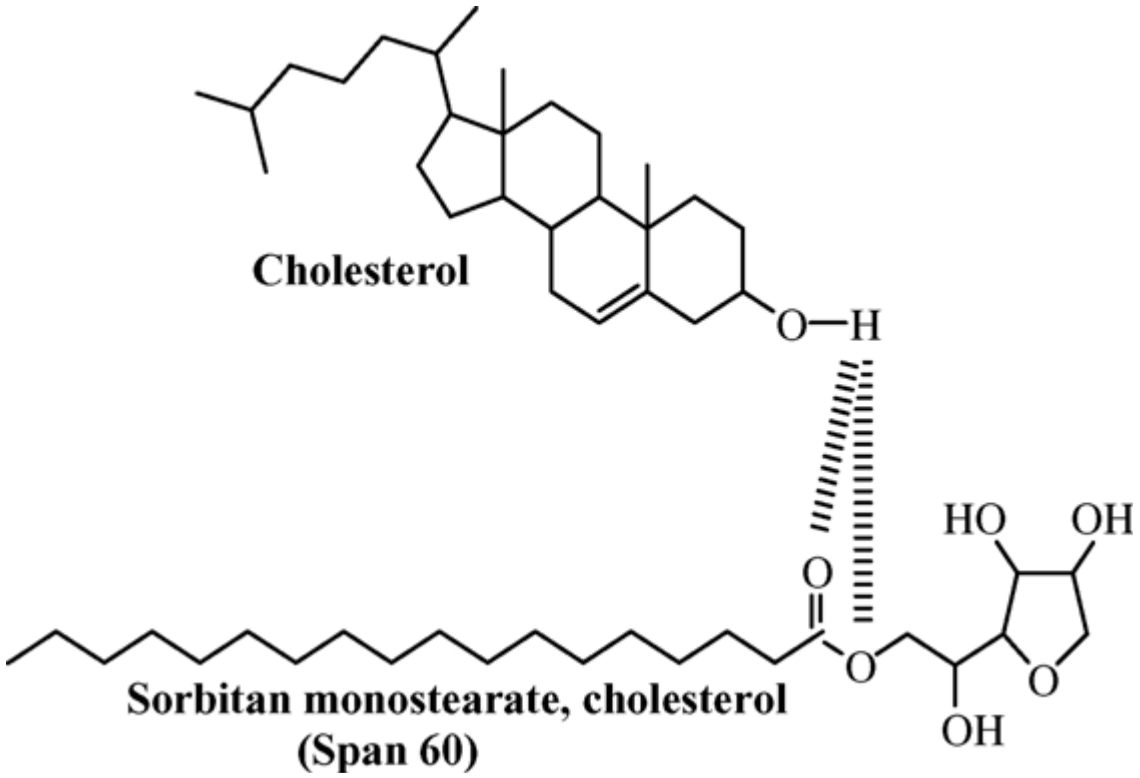


Figure: 9 ***Schematic showing the possible hydrogen bonding interaction between the β-OH group of the cholesterol and the oxygen primarily at the ketone group and also weaker interaction at the ester group.*** [63]

It is possible that the small hydrophilic 3 β-hydroxyl (β-OH) head group of the cholesterol in the bilayers is able to position itself in the vicinity of the sorbitan monostearate ester group and the hydrophobic steroid ring orients itself parallel to the acyl chains of the non-ionic surfactant. This may in effect restrict the movement of the acyl chains of the bilayer.

As shown in figure: 9 the β-OH group of the cholesterol could form a hydrogen bond with the oxygen at the ester group of the sorbitan monostearate. However, it is also possible to form hydrogen bonds at the other oxygen functionalities of sorbitan monostearate, which enhance the stability of the bilayer. These interactions result in an increase in membrane cohesion, as shown by increase in the mechanical stiffness of the membranes. There is only one possible hydrogen bonding group on the cholesterol moiety and suggest that the equimolar mixture represents the critical composition at which the two compounds can have extensive interaction at any of the mentioned sites.

According to Finean (1990) excessive concentration of cholesterol can cause their cluster formation leading to non-uniform distribution along the bilayers affecting the integrity of the membrane. Our studies suggest that a similar scenario may also pertain in niosomal membranes.

Effect of non-ionic surfactants on entrapment efficiency:

From the above results, F1 has higher entrapment efficiency compared with increasing concentration of surfactant while cholesterol content was maintained at a constant value. It indicates that increasing surfactant concentration leads to decrease in entrapment efficiency of the formulation.

The entrapment efficiency of various nonionic surfactants increases in the order of

35.05 % (F1 1:1) > 33.54 % (F8 1:1) > 29.42 % (F7 1:1) > 26.05 % (F9 1:1) > 22.05 % (F10 1:1).

These results explained that the Span 60 has higher entrapment efficiency than other Span types and Tween 60. This could be due to variation in the surfactant chemical structure. All span types have the same head group and different alkyl chain. Increasing the alkyl chain length is leading to higher entrapment efficiency. The entrapment efficiency followed the trend **Sp 60 (C18) > Sp 40 (C16)>Sp 20 (C12)>Sp 80 (C18)**. Sp 60 and Sp 80 have the same head groups but Sp80 has an unsaturated alkyl chain. De Gier et al. (1968) demonstrated that the introduction of double bonds into the paraffin chains causes a marked enhancement of the permeability of liposomes, possibly explaining the lower entrapment efficiency of the Sp 80 formulation. In addition, table no: 3 shows the phase transition temperatures of the Spans.

Table no: 3

S.No	Span type	Phase Transition temperature(°C)
1.	Span 60	53
2.	Span 40	42
3.	Span 20	16
4.	Span 80	-12

From the table Sp 80 has the lowest transition temperature amongst all tested spans (Kibbe, 2000). The span having the highest phase transition temperature provides the highest entrapment for the drug and vice versa. [4, 5]

SPAN 60 > SPAN 40 > SPAN 20 > SPAN 80 > TWEEN 60

The order of entrapment efficiency increased as the liphophilicity increased (HLB value decreased). Span 80 has the lowest HLB value but it has an unsaturated alkyl chain in its structure leads to lower entrapment efficiency.

Table no: 4

S.No.	Name	HLB value
1.	Span 20	8.6
2.	Span 40	6.7
3.	Span 60	4.7
4.	Span 80	4.3
5.	Tween 60	14.9

IN VITRO RELEASE STUDIES

The in vitro drug release study of ramipril loaded niosomes was done using dialysis bag diffusion technique in phosphate buffer saline pH 7.4. Figure: 13, 14 and table no 10, 11.

In vitro release from niosomes

The cumulative % drug release at 12 hours was 54.5 % for formulation F1 whereas it was 63.8 %, 66.7 %, 70.8 %, 79.9 %, 65.4 %, 77.4 %, 61.4 %, 62.2 % and 94.4 % for formulations F2, F3, F4, F5, F6, F7, F8, F9 and F10, respectively. Significant changes in release were observed upon changing the type of surfactant used in the bilayer of ramipril niosomes. In niosomal formulations the experimental studies showed that the rate of drug release depends on the percentage of drug entrapment efficiency. This result was in conformity with the report of "Samar Mansour"[65]

Generally, all niosome formulations showed significant slower release than ramipril solution (0.5mg/ml). This confirms that a sink condition for ramipril release was

accomplished and the dialysis bag used in the dissolution procedure does not limit ramipril release. Niosomal ramipril formulations with Span 60, Span 40, Span 20, Span 80 and Tween 60 show significant reduction in *invitro* drug release in 12hours compared with pure drug in solution.

Effect of surfactants on the release rate of ramipril from niosomes:

From the release studies of F1-F6, F1 showed the slower and prolonged drug release than the other formulations. This is due to higher entrapment efficiency. Further the release studies of the formulations containing Span 20, 40, 80 and Tween 60 are compared. It showed the release rate as the following order,

Tween 60 > Span 20 > Span 80 > Span 40 > Span 60

F10 (94.4%) > F7 (77.4%) > F9 (62.2%) > F8 (61.4%) > F1 (54.5%)

There is no significant difference between the release rates of Span 80 and Span 40 formulations. The entire amount of loaded drug was not released from the niosomes. This may be due to entrapment of the drug in the lipophilic region.

Comparison of In vitro drug release of ramipril loaded niosomes with ramipril pure drug solution:

The release of ramipril from niosomes is much slower and controlled than the ramipril pure drug solution (0.5mg/ml). Ramipril showed a release of about 96.4 % within 3.5 hours. The sustained release profile of niosomes was compared with pure drug solution and shown in table no: 12 and figure: 13, 14.

OPTIMIZATION OF PROCESS RELATED VARIABLES:

Determination of Viscosity [54]

The viscosity of the selected formulation Span 60 1:1 (30µmol) was determined using Ostwald's viscometer. The viscosity of the formulation was found to be 1.15 centipoises.

Effect of Sonication Time [54]

The niosomal formulation containing Span 60 1:1 (30µmol) is subjected to study the effect of sonication time, for various time intervals (like 1min, 2mins, 3mins, 4mins and 5mins). Particle size and entrapment efficiency of the formulation are showed at table no: 13 and figure 15 a-f.

The mean particle size and its distribution were measured by dynamic light scattering technique. From the figures it showed that the sonication time 3 minutes yield a smaller particle size compared with the other sonicated samples. Similar results also obtained with the entrapment efficiency. Therefore the optimal sonication time to formulate niosomal vesicles was found to be **3minutes.**

Effect of Hydration Time [54]

The niosomal formulation containing Span 60 1:1 (30µmol) is hydrated with 5.5 ml of PBS pH-7.4, for 30 minutes, 45minutes, 60minutes, 75minutes and 90minutes. The entrapment efficiency of the formulations is showed at the table no: 14. From the results hydration time of 45 minutes leads to higher entrapment efficiency. Therefore the optimal hydration time to formulate niosomal vesicles was found to be **45 minutes.**

Effect of Osmotic Shock [54, 55]

Formulation was treated with hypotonic (0.5% NaCl), hypertonic (0.9% NaCl), or normal saline (0.9% NaCl) solutions. The effect of osmotic shock is shown in figure no: 16 Increase in vesicle size was observed in formulation significantly in formulation incubated with hypotonic solution. In hypertonic solution, the formulation shrank uniformly. Formulations incubated with saline showed a slight increase in vesicle size. This demonstrates that ramipril niosomes could be diluted with normal saline for parenteral use.

Effect of Rotational Speed of Evaporator Flask [54]

The thickness and uniformity of the film depended upon the rotational speed of the flask. A speed of 100 rpm yielded a uniformly thin lipid film resulting in spherical vesicles on hydration. Lower and higher rpm (75 and 150 rpm) produced thick films that formed aggregates of vesicles on hydration.

Effect of charge inducing agents [56]

Niosomes were formulated with various amounts of the charge inducing agents, Stearylamine - STR (5 µmol), and Dicetylphosphate - DCP (5 µmol, 10 µmol and 15 µmol). The inclusion of a charge inducing agent in the lipid layer prevents the aggregation and fusion of vesicles, and maintains their integrity and uniformity. The optimal concentration of DCP in niosomes was identified based on entrapment efficiency. It was found that 10µmol DCP produced spherical vesicles with increased drug entrapment and without aggregation as shown in table no: 15.

Role of Charge-inducing Agents in Drug Entrapment:

The effect of charge on niosome bilayers is a matter of debate. Some reports have concluded that negatively charged liposomes are more effective in drug entrapment than neutral liposomes. The effect of STR and different micromolar concentrations of DCP on percentage entrapment of Span 60 formulation is shown in Figure: 12. Formulations with 5µmol STR show good entrapment at a surfactant/cholesterol ratio of 1: 1. Formulations of Span with DCP decreased drug entrapment. In Span 60 formulation, DCP decreased entrapment whereas in formulation containing STR, entrapment was increased table no: 16**.** These results show that inclusion of DCP alters the entrapment; it depends upon the alkyl side-chain of the surfactant.

The inclusion of DCP into ramipril niosomes was found to decrease percentage entrapment efficiency with respect to the control formulation (F1- without charge inducing agent) which was presumably due to the electrostatic repulsion forces between the carboxyl group of ramipril and an anionic head group of DCP. In contrast, the inclusion of STR into ramipril niosomes at appeared to increase percentage entrapment efficiency of ramipril greater than the control formulation (F1- without charge inducing agent), owing to the electrostatic attraction between the positively charged head group of STR and the carboxyl group of ramipril [56].

Role of Charge-inducing Agents in Drug Release Rate:

The in vitro drug release study of F1, F11 and F12 was done using dialysis bag diffusion technique in phosphate buffer saline pH 7.4. The drug release rate at 12hours for F1 was 54.5%, F11 was 58.5% and F12 was 67.4% [table no: 17]. Inclusion of DCP in Span 60 formulation increased the percent release of drug and extended the time of release compared with formulation without DCP (Figure: 17, 18, 19). Similar results were reported for Span 40 and Span 60 by Manconi et al.

Zeta potential measurement:

The zeta potential of the formulation (F12) containing 10 µmol DCP was measured using Malvern Zeta Sizer using disposable sizing cuvette. The particle size and the charge on the vesicle membrane are shown in figure 20a, 20b & 21. The average particle size was found to be **256.9nm**. The Poly Dispersibility Index (PDI) was found to be 0.494. The zeta potential of the F12 formulation was found to be **-84.3mV.**

KINETICS OF DRUG RELEASE [50, 54]

Linear regression analysis for the release was done to determine the proper order of release. From the results shown in table no: 18. Tween 60 formulation follows zero-order kinetics and the other formulations obey first-order kinetics. Calculation of Higuchi's correlation coefficient confirms that drug release was proportional to the square root of time indicating that ramipril release from niosomes was diffusion controlled. The n value from the Korsmeyer-Peppas model for ramipril niosomal formulation was between 0.728 and 0.99 which confirms the Non-Fickian type diffusion, follow an anomalous diffusion mechanism with erosion (n>0.54). Release profiles also fitted into a Hixson-Crowell model and confirmed that drug release from niosomes followed anomalous diffusion. The similar results were obtained by Ahmed A Guinea et al and Alok Namdeo et al. The drug release pattern from ramipril loaded niosomes follows **Higuchi's model and first order of release.**

SCANNING ELECTRON MICROSCOPY (SEM)

The particle size, shape and surface morphology was evaluated using SEM. SEM photographs of ramipril loaded niosome of selected formulations F1was observed. In the selected formulation the particles were almost spherical and homogenous. The micrographs also confirmed that niosomes was in nanometer size. The results showed that the ramipril loaded niosomes particles have a spherical shape with smooth surface. This is shown in the figure: 22.

FT - IR STUDIES

FT-IR studies were carried out to confirm the compatibility between drug ramipril, the non-ionic surfactants used (Span 20, Span 40, Span 60, and Span 80), cholesterol and niosome formulation. The spectra obtained from the FT-IR studies are from 4400 cm^{-1} to 450 cm^{-1}.

The FT-IR spectra of the formulations are shown in Figure: 23, 24 a,b, 25 a,b, 26 a,b, & 27 a,b. It was confirmed that there are no major shifting as well as any loss of functional peaks between the spectra of drug, non- ionic surfactant, cholesterol and drug loaded niosome. [73]

The FT-IR spectra of ramipril drug shows as follows:

Table No: 5

S.No	Wave Number (cm^{-1}).	Bond
1.	3280	-CH stretching, -NH stretching, -OH stretching
2.	3200-3400	-OH bond (H bonded alcohols and phenols)
3.	2850-2960	Alkanes
4.	1680-1760	Aldehydes, Ketones, Carboxylic acids
5.	1600-1700	-C-C stretching, C=N stretching
6.	1500-1700	-NH bending
7.	1500-1600	C=C (Aromatic rings)
8.	1180-1360	-C-N amines
9.	1200-1500	-OH bending
10.	700-900	-NH rocking

DIFFERENTIAL SCANNING CALORIMETRY (DSC Studies) [60]

DSC studies are useful method of detecting drug-excipient incompatability. DSC thermograms of the pure drug (Ramipril), Non-ionic surfactants (Span 40 and Span 60), cholesterol and formulations are shown in the figure: 28.

Drug showed the sharp melting endothermic peak at 109º C. Span 40 showed at 48º C and Span 60 showed at 57º C. It suggests that the formulation components Span 60, Span 40, cholesterol and the drug ramipril do not interact to form any additional chemical entity but remain as mixture.

STABILITY OF RAMIPRIL LOADED NIOSOMES:

The stability studies of the selected formulation of ramipril loaded niosomes was carried out by storing at 4^0C (refrigeration temperature) and 25^0C $\pm$ 2^0C [2, 15].

The entrapment efficiency of the drug in the niosomal dispersion was estimated immediately after the preparation and the results are shown in the table no: 19. The entrapment efficiency of the niosomal dispersion, after every week was estimated and the results are shown in the table no: 19 and figure 29.

From the table no: 19 and figure: 29, entrapment efficiency of the formulation stored at refrigerated temperature (4±2^0C/60% RH) showed only a slight decrease after 1month compared to the formulation stored at (25^0C $\pm$ 2^0C/60% RH). Hence increase in temperature and storage period decreases the entrapment efficiency of niosomal dispersion irrespective of the non-ionic surfactants used for formulations. Comparatively slightly high entrapment efficiency of drug was observed with niosomes stored in refrigerated temperature.

CHAPTER XII
SUMMARY AND CONCLUSION

- ✓ The purpose of this research was to prepare ramipril loaded niosomes for controlled release of drug and a trial to improve the bioavailability.
- ✓ Thin film hydration technique was employed to produce niosomes using non ionic surfactants and cholesterol.
- ✓ Preformulation studies were conducted to fix the cholesterol concentration used in our formulations. From the results it was concluded that 30µmol concentration of cholesterol was fixed and used in all formulations.
- ✓ Cholesterol was used as a membrane additive, acts as a stabilizer as well as fluidity buffer to improve the stability of the vesicles.
- ✓ The formulations were prepared by varying the surfactant concentration (Span 60) and cholesterol concentration fixed at constant value.
- ✓ The formulated niosomes were characterized for entrapment efficiency and in vitro release studies in phosphate buffered saline pH 7.4.
- ✓ The niosomal drug delivery system of ramipril prepared from Span 60 equimolar mixture (1:1) showed better entrapment efficiency and sustained drug release.
- ✓ This ratio (1:1) was used to prepare the formulations by using other non-ionic surfactants (Span 40, Span 20, Span 80 and Tween 60).
- ✓ The better entrapment efficiency of niosomes was obtained with Span 60 about 35.05 % due to the higher lipophilicity (HLB value- 4.7) of the surfactant and the highest phase transition temperature.
- ✓ The order of entrapment efficiency as follows;
  SPAN 60 > SPAN 40 > SPAN 20 > SPAN 80 > TWEEN 60
- ✓ The order of *invitro* release study as follows;
  Tween 60 > Span 20 > Span 80 > Span 40 > Span 60

The sustained drug release from the Span 60 is due to entrapment of the drug in the lipophilic region.

- ✓ The release of ramipril from niosomes is much slower and controlled than the ramipril pure drug solution (0.5mg/ml).
- ✓ The process related parameters were optimized such as sonication time (3 mins), hydration time (45 mins), osmotic shock, rotational speed of the evaporator flask (100 rpm), and the effect of charge inducing agents such as STR and DCP.
- ✓ The inclusion of STR into ramipril niosomes at appeared to increase %E.E. of ramipril due to the electrostatic attraction between the positively charged head group of STR and the carboxyl group of ramipril.
- ✓ The inclusion of DCP into ramipril niosomes was found to decrease %E.E. due to the electrostatic repulsion forces between the carboxyl group of ramipril and an anionic head group of DCP.
- ✓ The zeta potential of the formulation containing DCP 10µmol (F12) was found to be - **84.3mV.**
- ✓ The drug release pattern from ramipril loaded niosomes follows **Higuchi's model and first order of release.**
- ✓ The particle size of the formulated ramipril niosomes exhibited nanometer size range spherical shape particles. SEM analysis of the niosomes dispersion showed the spherical shape of the vesicles.
- ✓ The results of the FT-IR studies and DSC studies proved that no interactions between the drug, cholesterol, non-ionic surfactants and formulations.
- ✓ Stability studies indicated that the entrapment efficiency of the niosomes was not affected significantly in the refrigerated storage temperature. However there may be a slight reduction in the entrapment efficiency of the niosomes due to the drug expulsion from the vesicles.

✪ It is concluded that the thin film hydration technique, is a useful method for the successful incorporation of poorly water soluble drug ramipril into niosomes with high entrapment efficiency. The prolonged release of the drug from the niosomes suggests that the frequency of administration may be reduced. Further it may be presumed that if the nanometer range particles are obtained, the bioavailability may be increased. Further investigations in animals, human volunteers, pharmacological and toxicological investigations in animals and human volunteers may help to exploit the niosomes as prosperous drug carriers for targeting drugs more efficiently. Hence we can conclude that niosomes provide controlled release of drug and these systems are used as drug carriers to enhance the bioavailability of poorly water soluble drugs.

Table No-6

CALIBRATION CURVE OF RAMIPRIL IN

PHOSPHATE BUFFERED SALINE pH 7.4.

S.No.	CONCENTRATION (µg/ml)	ABSORBANCE ± SD*
1	5	0.196 ± 0.0005
2	10	0.390 ± 0.007
3	15	0.587 ± 0.0057
4	20	0.781 ± 0.0065
5	25	0.974 ± 0.0045
6	30	1.182 ± 0.0091
7	35	1.382 ± 0.0043
8	40	1.564 ± 0.015
9	45	1.747 ± 0.016
10	50	1.972 ± 0.0087

n = 3*

γ = 0.999901195

Table No: 7
ENTRAPMENT EFFICIENCIES OF RAMIPRIL NIOSOMES USING SPAN 60: CHOLESTEROL (1:1) AT DIFFERENT MOLAR CONCENTRTIONS.

S.NO	MOLAR CONCENTRATION	% ENTARPMRNT EFFICIENCY ± SD*
1	20 µmol	32.28 ± 0.33
2.	30 µmol	35.30 ± 0.17
3.	40 µmol	34.11 ± 0.58
4.	50 µmol	30.62 ±0.69
5.	60 µmol	28.33 ± 0.47
6.	70 µmol	25.94 ± 0.28

n=3*

Table No: 8

FORMULATION OF RAMIPRIL NIOSOMES

S.NO	FORMULATION	SURFACTANT	RATIO OF	
			SURFACTANT	CHOLESTEROL
1.	F1	SPAN 60	1	1
2.	F2	SPAN 60	2	1
3.	F3	SPAN 60	3	1
4.	F4	SPAN 60	4	1
5.	F5	SPAN 60	5	1
6.	F6	SPAN 60	6	1
7.	F7	SPAN 20	1	1
8.	F8	SPAN 40	1	1
9.	F9	SPAN 80	1	1
10.	F10	TWEEN 60	1	1

Drug concentration used in each formulation kept as constant 2.5mg/5ml.

In ratio 1 stands for 30µmol.

Table No: 9

% ENTRAPMENT EFFICIENCY OF DIFFERENT FORMULATIONS

S.NO	FORMULATION	SURFACTANT	RATIO OF		% ENTRAPMENT EFFICIENCY ± SD*
			SURFACTANT	CHOLESTEROL	
1.	F1	SPAN 60	1	1	35.05±0.46
2.	F2	SPAN 60	2	1	30.76±0.65
3.	F3	SPAN 60	3	1	29.16±0.56
4.	F4	SPAN 60	4	1	28.06±0.71
5.	F5	SPAN 60	5	1	27.57±0.39
6.	F6	SPAN 60	6	1	25.28±0.50
7.	F7	SPAN 20	1	1	29.42±0.57
8.	F8	SPAN 40	1	1	33.54±0.65
9.	F9	SPAN 80	1	1	26.05±0.70
10.	F10	TWEEN 60	1	1	22.05±0.58

n=3*

Table No: 10

COMPARISON OF INVITRO RELEASE PROFILE OF RAMIPRIL NIOSOMES

TIME IN HOURS	CUMULATIVE % DRUG RELEASE ± SD*				
	F1 (SPAN 60 1:1)	F2 (SPAN 60 1:2)	F3 (SPAN 60 1:3)	F4 (SPAN 60 1:4)	F5 (SPAN 60 1:5)
0.00	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0
0.25	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
0.5	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
0.75	0 ± 0	3.7 ± 1.80	4.7 ± 0.81	4.8 ± 0.51	5.5 ± 0 .61
1.0	4.1 ± 0.24	7.4 ± 1.09	8.2 ± 1.02	7.7 ± 0.99	8.6 ± 1.32
1.5	7.7 ± 1.71	11.5 ± 0.87	12.3 ± 0.98	13.2 ± 0.69	13.3 ± 1.00
2.0	11.3 ± 1.75	15.7 ± 0.96	16.1 ± 1.84	17.6 ± 1.59	18.4 ± 1.00
2.5	15.9 ± 1.67	19.6 ± 1.46	21.4 ± 1.51	22.6 ± 2.09	25.5 ± 1.49
3.0	19.7 ± 2.54	23.8 ± 1.07	24.3 ± 2.03	26.8 ± 1.88	30.9 ± 1.48
3.5	24.5 ± 2.01	26.5 ± 2.48	29.8 ± 1.42	30.0 ± 2.00	34.9 ± 1.79
4.0	26.7 ± 1.76	29.7 ± 0.75	32.1 ± 1.52	31.7 ± 1.45	39.3 ± 2.20
4.5	29.3 ± 1.10	31.7 ± 1.59	35.5 ± 1.70	34.1 ± 0.70	42.6 ± 1.29
5.0	31.4 ± 1.95	37.0 ±1.62	38.3 ± 1.65	36.1 ± 1.29	44.1 ± 0.89
5.5	33.9 ± 0.60	39.7 ± 0.40	41.1 ± 0.96	40.7 ± 1.29	47.8 ± 1.29
6.0	34.8 ± 0.40	42.6 ± 1.49	42.6 ± 0.47	45.6 ± 1.30	51.0 ±2.09
6.5	37.2 ± 1.04	46.6 ± 1.85	46.0 ± 1.18	49.6 ± 1.30	56.5 ± 0.52
7.0	39.2 ± 0.94	48.6 ± 1.34	50.2 ± 1.07	52.3 ± 0.65	56.9 ± 0.22
7.5	42.6 ± 0.58	50.8 ± 1.17	53.7 ± 0.94	57.0 ± 1.97	60.8 ± 1.89
8.0	44.0 ± 1.16	53.1 ± 1.17	56.0 ± 1.95	61.4 ± 0.83	64.1 ± 0.52
9.0	46.5 ± 1.28	54.7 ± 1.60	58.4 ± 0.96	65.8 ± 0.95	68.1 ± 1.29
10.0	49.7 ± 1.19	59.1 ± 1.10	61.1 ± 1.15	67.0 ± 0.19	71.4 ± 2.39
11.0	52.1 ± 0.02	60.7 ± 1.00	63.2 ± 1.98	69.6 ± 1.05	74.9 ± 1.39
12.0	54.5 ± 0.65	63.8 ± 1.50	66.7 ± 1.57	70.8 ± 1.39	79.9 ± 0.70

n=3*

Table No: 11

COMPARISON OF INVITRO RELEASE OF RAMIPRIL NIOSOMES

TIME IN HOURS	CUMULATIVE % DRUG RELLEASE ± SD*				
	F6(SPAN 60 1:6)	F7(SPAN 20 1:1)	F8(SPAN 40 1:1)	F9(SPAN80 1:1)	F10(TWEEN60 1:1)
0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
0.25	0 ± 0	5.0 ± 0.42	4 ± 0.51	4.1 ± 0.80	4.6 ±0.22
0.5	0 ±0	7.0 ± 0.69	5.9 ± 1.51	6.2 ± 1.18	6.8 ±1.49
0.75	5.2 ±0 .99	10.6 ± 1.39	8.5 ± 1.16	8.2 ± 2.30	9.3 ±1.03
1.0	8.3 ± 1.29	15.3 ± 1.14	11.6 ± 1.14	10.9 ± 1.65	14.0 ±1.09
1.5	11.4 ± 0.59	19.5 ± 1.69	14.0 ± 1.72	15.0 ± 1.25	18.8 ±2.50
2.0	14.6 ± 1.59	23.8 ± 0.59	17.0 ± 1.17	18.1 ± 1.53	23.3 ±1.34
2.5	18.5 ± 1.69	28.4 ± 0.99	19.7 ± 1.16	21.0 ± 1.26	28.2 ±1.28
3.0	22.3 ± 1.99	33.2 ± 0.71	23.7 ± 1.19	23.6 ± 1.98	32.0 ±1.29
3.5	25.6 ± 2.39	36.5 ± 1.30	26.7 ± 1.00	26.0 ± 1.26	36.2 ±1.19
4.0	28.9 ± 1.42	39.1 ± 1.32	30.0 ± 1.20	29.2 ± 1.04	40.8 ±1.34
4.5	32.2 ± 2.21	42.2 ± 0.61	32.4 ± 1.23	32.1 ± 1.03	45.5 ±1.92
5.0	35.9 ± 3.89	44.5 ± 0.91	34.3 ± 1.19	35.6 ± 2.01	49.9 ±1.27
5.5	39.4 ± 2.89	48.7 ± 1.80	38.0 ± 1.15	38.7 ± 1.76	54.5 ±1.08
6.0	42.1 ± 1.51	51.1 ± 1.48	40.5 ± 1.13	40.9 ± 1.26	57.7 ±1.25
6.5	46.2 ± 1.21	53.9 ± 1.23	43.9 ± 1.86	43.8 ± 1.25	61.2 ±1.20
7.0	48.9 ± 1.41	57.4 ± 2.01	46.8 ± 1.26	46.7 ±1.20	65.6 ±0.74
7.5	51.8 ± 0.71	59.9 ± 1.74	49.5 ± 0.01	48.8 ±1.92	70.3 ±1.79
8.0	53.7 ± 0.80	62.8 ± 0.94	51.5 ± 1.24	50.9 ±1.95	74.6 ±1.02
9.0	56.4 ± 1.10	67.4 ± 1.17	52.7 ± 0.25	52.6 ±1.17	79.5 ±1.09
10.0	58.9 ± 1.31	70.4 ± 2.11	55.5 ± 0.92	55.8 ±1.10	84.0 ±1.03
11.0	62.7 ± 1.69	73.9 ± 1.16	58.8 ± 1.62	58.2 ±1.49	88.2 ±1.04
12.0	65.4 ± 0.59	77.4 ± 1.18	61.4 ± 0.74	62.2 ±1.08	94.4 ±1.04

n=3*

Table No: 12
COMPARISON ON INVITRO RELEASE OF RAMIPRIL NIOSOMES (SPAN 60 1:1) WITH RAMIPRIL DRUG SOLUTION

TIME IN HOURS	CUMULATIVE % DRUG RELEASE±SD*	
	F1 (SPAN 60 1:1)	PURE DRUG SOLUTION
0	0 ± 0	0 ± 0
0.25	0 ± 0	30.3 ± 0.83
0.5	0 ± 0	43.3 ± 1.40
0.75	0 ± 0	48.6 ± 1.31
1.0	4.1 ± 0.24	54.9 ± 0.70
1.5	7.7 ± 1.71	62.3 ± 1.69
2.0	11.3 ± 1.75	68.1 ± 1.59
2.5	15.9 ± 1.67	75.7 ± 1.49
3.0	19.7 ± 2.54	85.8 ± 2.96
3.5	24.5 ± 2.01	96.4 ± 2.78
4.0	26.7 ± 1.76	97.3 ± 2.67
4.5	29.3 ± 1.10	98.0 ± 1.08
5.0	31.4 ± 1.95	98.7 ± 1.05
5.5	33.9 ± 0.60	98.6 ± 1.13
6.0	34.8 ± 0.40	97.6 ± 0.79
6.5	37.2 ± 1.04	97.3 ± 0.45
7.0	39.2 ± 0.94	97.3 ± 0.67
7.5	42.6 ± 0.58	97.2 ± 1.56
8.0	44.0 ± 1.16	97.1 ± 0.57
9.0	46.5 ± 1.28	96.6 ± 0.94
10.0	49.7 ± 1.19	96.3 ± 1.47
11.0	52.1 ± 0.02	96.0 ± 1.18
12.0	54.5 ± 0.65	95.7 ± 0.60

n=3*

Table no: 13
EFFECT OF SONICATION TIME (SPAN 60 1:1)

S.NO	SONICATION TIME (MINUTES)	% ENTRAPMENT EFFICIENCY
1	0	35.90
2	1	38.64
3	2	38.92
4	3	47.22
5	4	42.51
6	5	36.54

Table no: 14 EFFECT OF

S.NO	HYDRATION TIME(MINUTES)	% ENTRAPMENT EFFICIENCY
1	30	35.22
2	45	52.21
3	60	41.63
4	75	31.04
5	90	13.12

Table No: 16

ENTRAPMENT EFFICIENCIES OF RAMIPRIL NIOSOMES WITH AND WITHOUT CHARGE INDUCING AGENTS.

S.NO	FORMULATION	% ENTRAPMENT EFFICIENCY ± SD*
1.	F1	35.05±0.46
2.	F11	40.53±0.33
3.	F12	27.88±0.48
4.	F13	20.45±0.88
5.	F14	20.62±0.56

n=3*

Table No: 15

FORMULATION OF RAMIPRIL NIOSOMES WITH AND WITHOUT CHARGE INDUCING AGENTS.

S.NO.	FORMULATION	SURFACTANT : CHOLESTEROL (1:1 MOLAR RATIO)	CHARGE INDUCING AGENTS	
			STR	DCP
1.	F1	30 µmol	--	--
2.	F11	30 µmol	5 µmol	--
3.	F12	30 µmol	--	10 µmol
4.	F13	30 µmol	--	5 µmol
5.	F14	30 µmol	--	15 µmol

Drug concentration used in each formulation kept as constant 2.5mg/5ml.

Table No: 18

DETERMINATTION OF ORDER OF RELEASE OF RAMIPRIL FROM NIOSOMAL FORMULATIONS

Formulation	Higuchi	Korsemeyer-Peppas		Zero order		First order		Hixson-Crowell		Release mechanism
	r^2	r^2	n	r^2	K_0 (% mg/h)	r^2	K_1 (h^{-1})	r^2	Slope (n)	
F1	0.9945	0.9581	0.9853	0.957	4.997	0.9885	0.0306	0.980	0.0968	NFD
F2	0.9926	0.9697	0.9533	0.960	5.800	0.9926	0.0392	0.986	0.1262	NFD
F3	0.9945	0.9777	0.9108	0.959	6.007	0.9938	0.0418	0.986	0.1196	NFD
F4	0.9869	0.9796	0.9369	0.968	6.559	0.9882	0.0487	0.987	0.1437	NFD
F5	0.9969	0.9749	0.9301	0.961	7.066	0.9971	0.0572	0.995	0.1625	NFD
F6	0.9917	0.9906	0.9066	0.969	5.921	0.9954	0.0405	0.991	0.1231	NFD
F7	0.9974	0.9939	0.7280	0.963	6.401	0.9984	0.0522	0.996	0.1067	NFD
F8	0.9897	0.9967	0.7341	0.968	5.206	0.9935	0.0350	0.988	0.1067	NFD
F9	0.9916	0.9977	0.7284	0.969	5.188	0.9951	0.0350	0.988	0.1071	NFD
F10	0.9876	0.9972	0.8157	0.984	8.003	0.9409	0.0876	0.988	0.2196	NFD

NFD- NONFICKIAN DIFFUSION

Table No: 17

COMPARISON ON INVITRO RELEASE OF RAMIPRIL NIOSOMES (SPAN 60 1:1) WITH AND WITHOUT CHARGE INDUCING AGENTS.

TIME IN HOURS	CUMULATIVE % DRUG RELEASE±SD*		
	F1	F11(STR)	F12(DCP)
0	0 ± 0	0 ± 0	0 ± 0
0.25	0 ± 0	1.10 ± 0.67	1.30 ± 1.51
0.5	0 ± 0	2.30 ± 0.96	2.60 ± 1.21
0.75	0 ± 0	3.90 ± 1.80	3.90 ± 0.80
1.0	4.1 ± 0.24	5.50 ± 1.09	8.20± 1.02
1.5	7.7 ± 1.71	8.30 ± 0.87	11.6 ± 0.98
2.0	11.3 ± 1.75	12.2 ± 0.96	15.8 ± 1.80
2.5	15.9 ± 1.67	14.6 ± 1.46	20.9 ± 1.53
3.0	19.7 ± 2.54	17.3 ± 1.07	23.8 ± 2.02
3.5	24.5 ± 2.01	19.5 ± 2.48	28.5 ± 1.40
4.0	26.7 ± 1.76	20.5 ± 0.75	33.4 ± 1.54
4.5	29.3 ± 1.10	22.6 ± 1.60	36.0 ± 1.72
5.0	31.4 ± 1.95	24.5 ± 1.60	39.0 ± 1.65
5.5	33.9 ± 0.60	27.8 ± 0.40	40.5 ± 0.96
6.0	34.8 ± 0.40	32.1 ± 1.50	42.6 ± 0.47
6.5	37.2 ± 1.04	35.7 ± 1.85	45.7 ± 1.18
7.0	39.2 ± 0.94	39.2 ± 1.34	49.8 ± 1.07
7.5	42.6 ± 0.58	42.2 ± 1.17	52.8 ± 0.94
8.0	44.0 ± 1.16	45.4 ± 1.00	56.8 ± 1.95
9.0	46.5 ± 1.28	49.0 ± 1.60	59.0 ± 0.96
10.0	49.7 ± 1.19	52.8 ± 1.10	60.3 ± 1.15
11.0	52.1 ± 0.02	55.3 ± 1.00	63.2 ± 1.98
12.0	54.5 ± 0.65	58.5 ± 1.50	67.4 ± 1.57

n=3* STR- Stearylamine DCP- Dicetyl phosphate.

Table no: 19

STABILITY STUDIES OF NIOSOMES CONTAINING SPAN 60 (1:1)

S.NO	WEEK	% ENTRAPMENT EFFICIENCY AT 60% RH±5% RH	
		4 ± 2^0C	25 ± 2^0 C
1.	0	35.05	35.05
2.	1	35.00	34.54
3.	2	34.88	33.21
4.	3	34.29	32.44
5.	4	33.81	31.07

Figure: 10 DETERMINATION OF λ_{max} OF RAMIPRIL IN PHOSPHATE BUFFERED SALINE PH- 7.4.

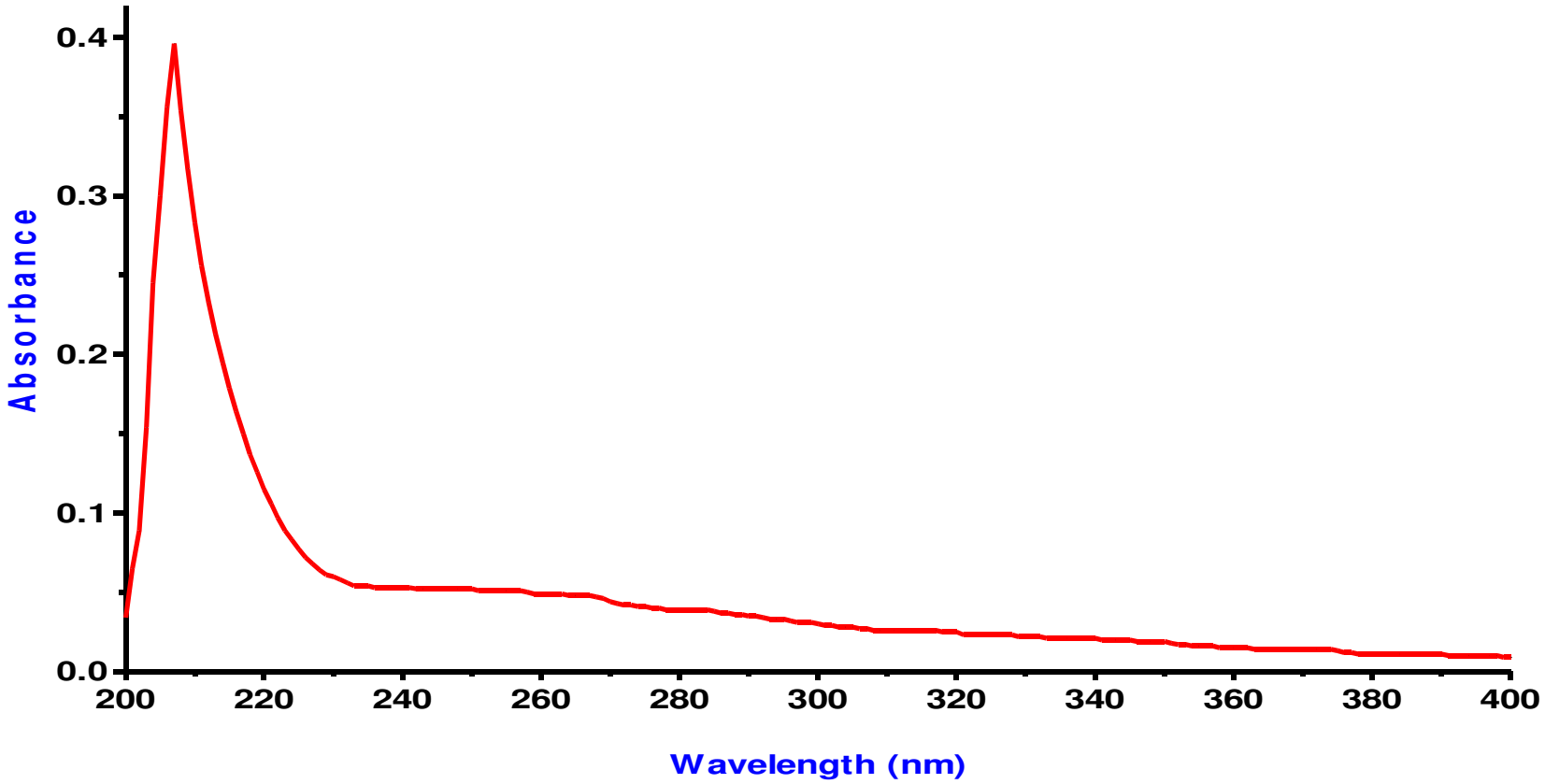


Figure- 11

CALIBRATION OF RAMIPRIL IN PBS (pH 7.4) AT λ_{max} 207nm

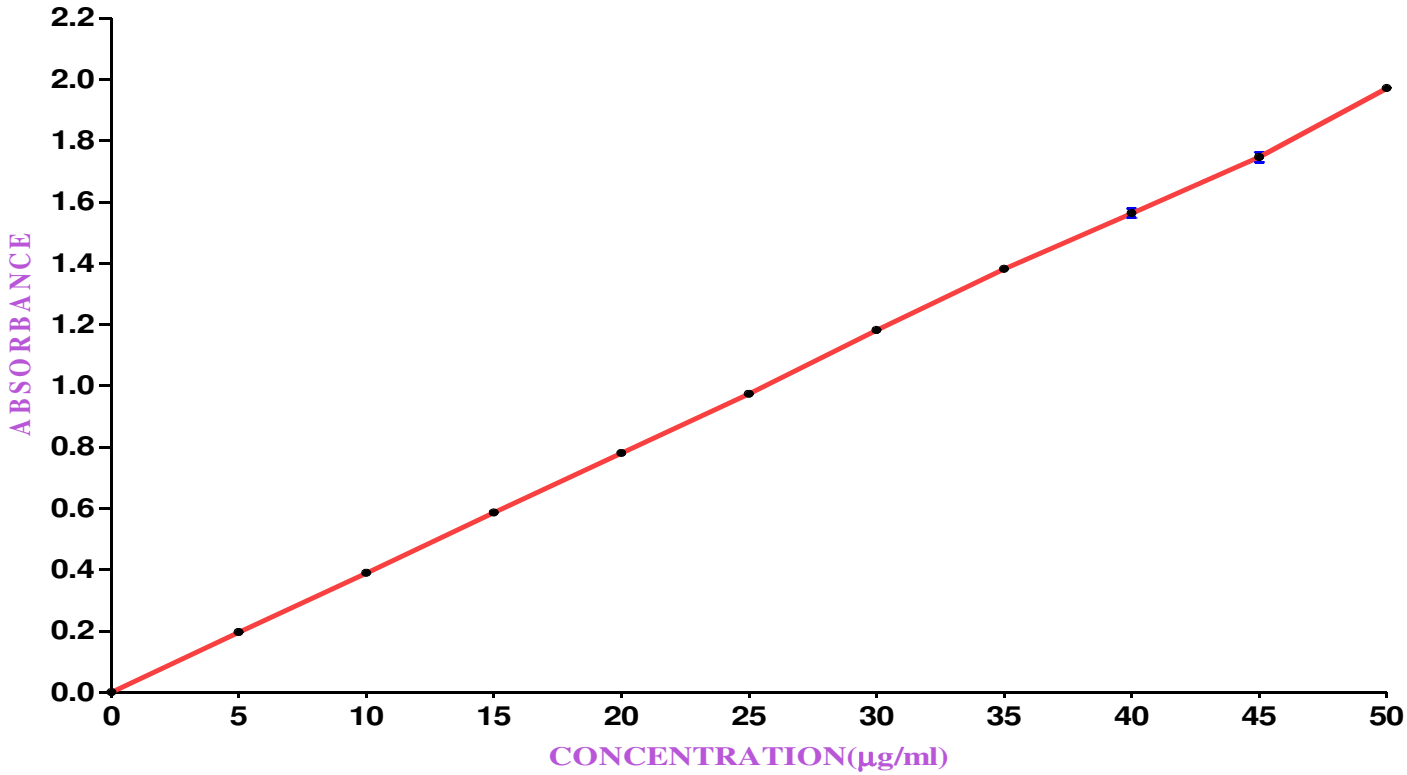


Figure- 12

COMPARISON OF ENTRAPMENT EFFICIENCIES OF RAMIPRIL NIOSOMES.

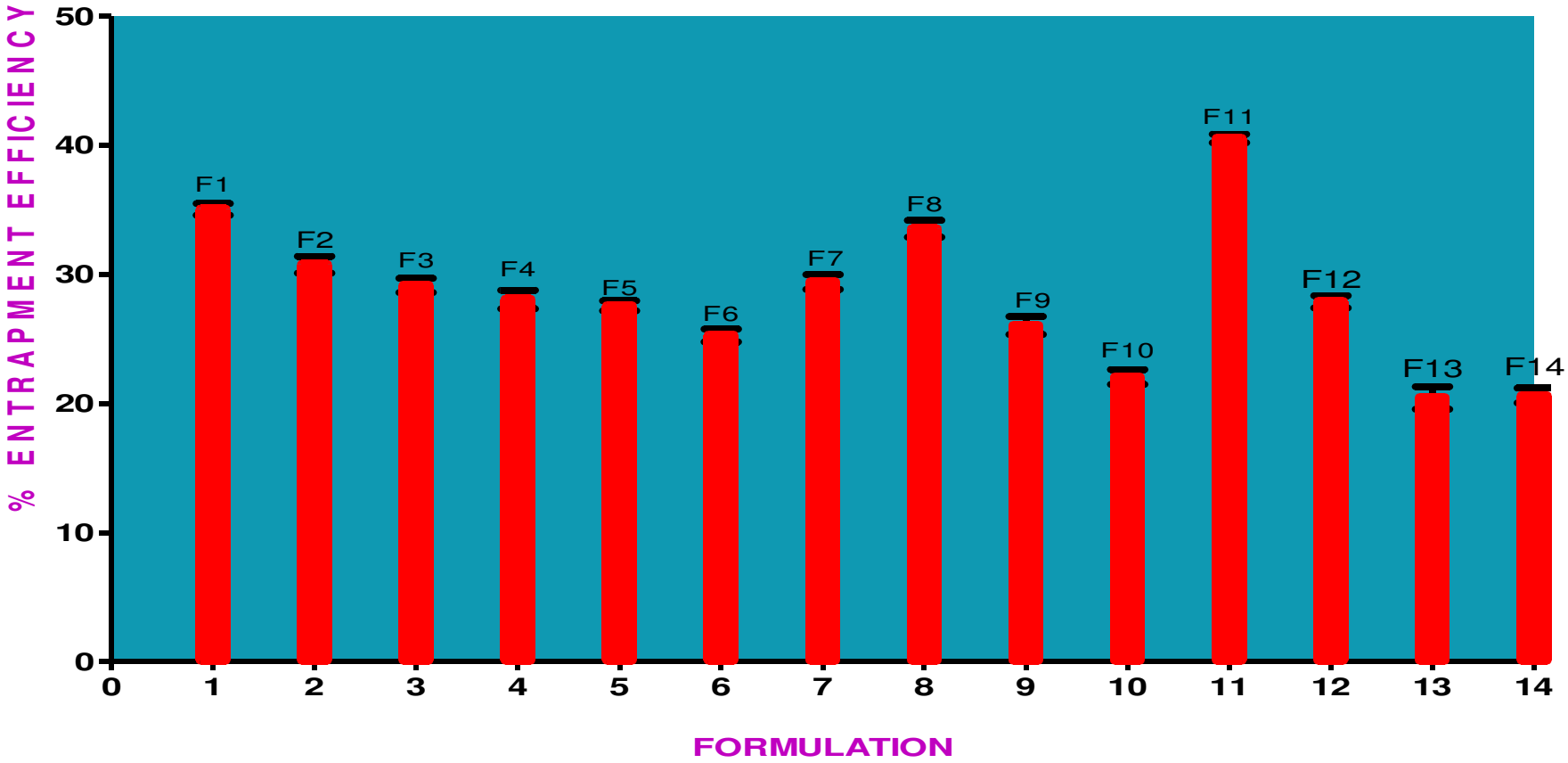


Figure- 13

COMPARISON OF INVITRO RELEASE OF RAMIPRIL NIOSOMES CONTAINING SPAN 60 AT DIFFERENT RATIOS.

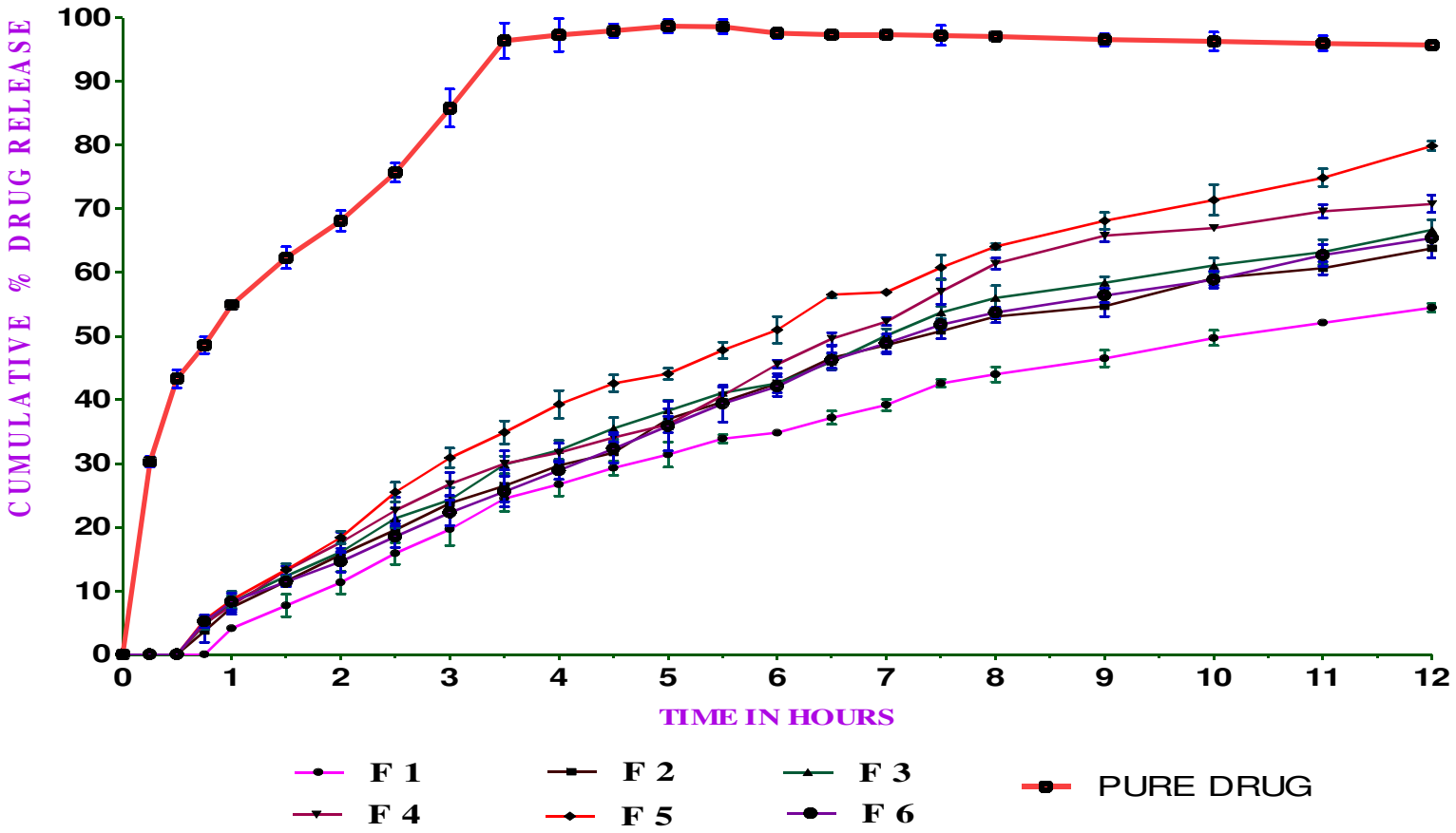


Figure: 15 a.

PARTICLE SIZE DISTRIBUTION F1

(SONICATION TIME= 0 MINUTES)

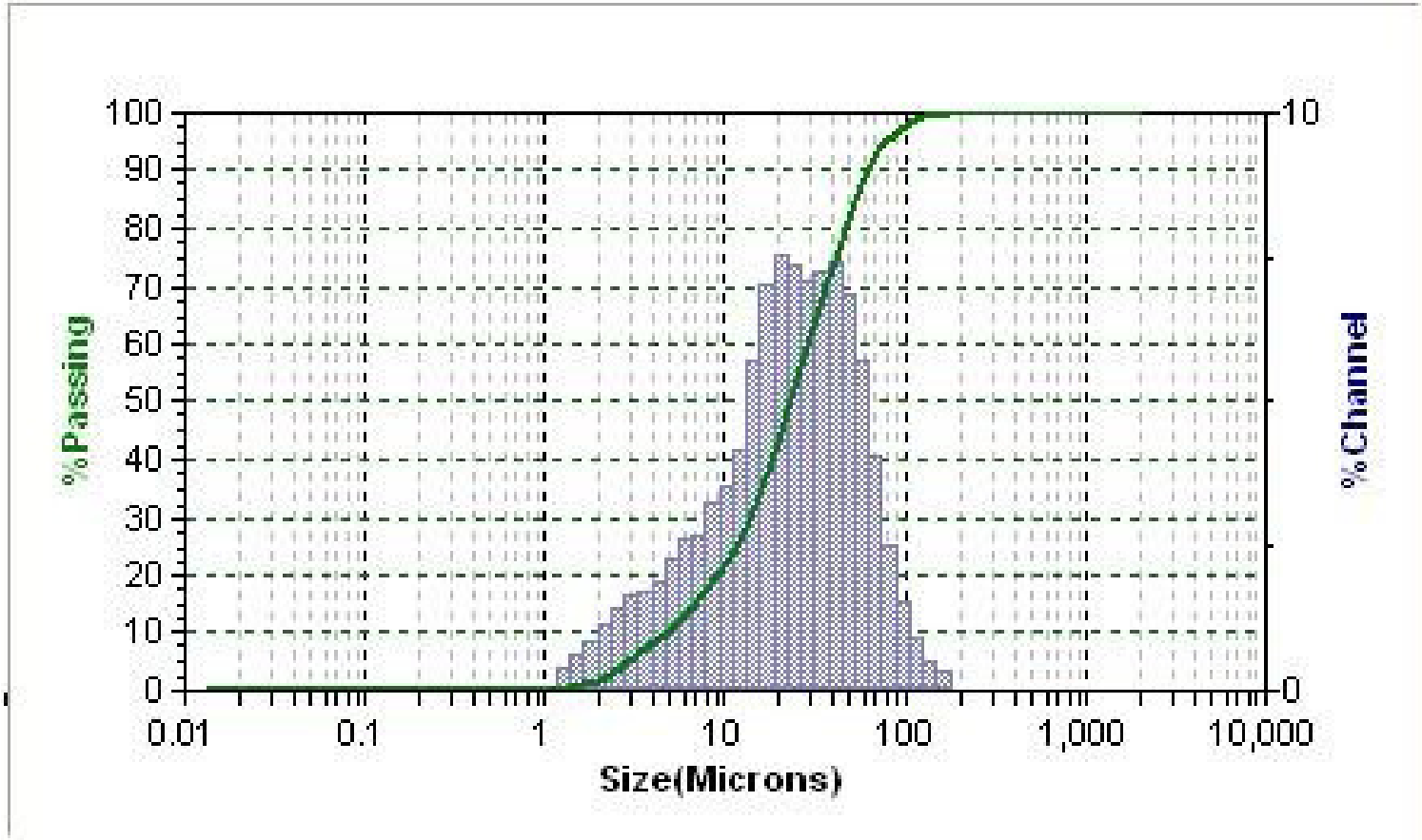


Figure: 15 b.

PARTICLE SIZE DISTRIBUTION F1

(SONICATION TIME= 1 MINUTE)

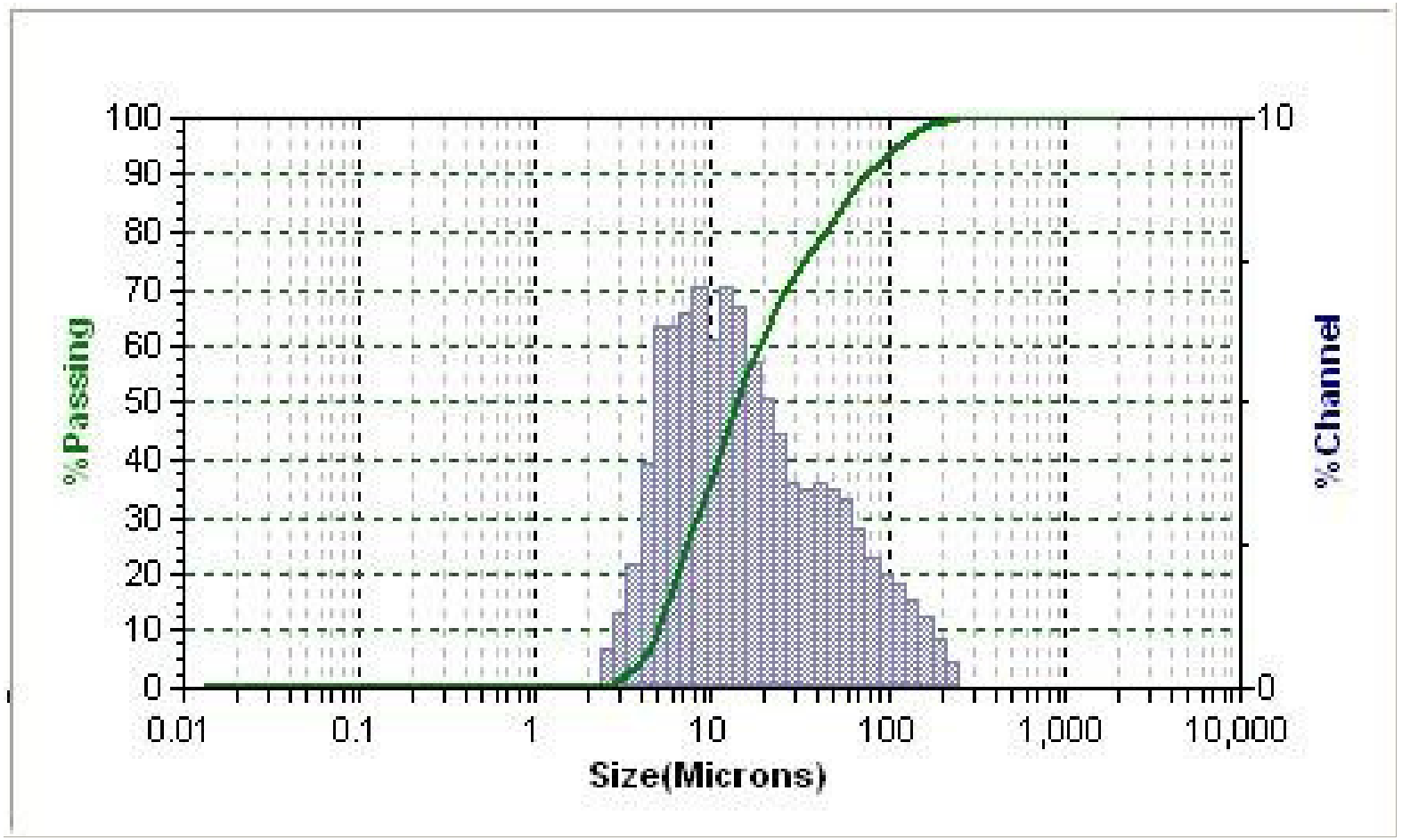


Figure: 15 c.

PARTICLE SIZE DISTRIBUTION F1

(SONICATION TIME= 2 MINUTES)

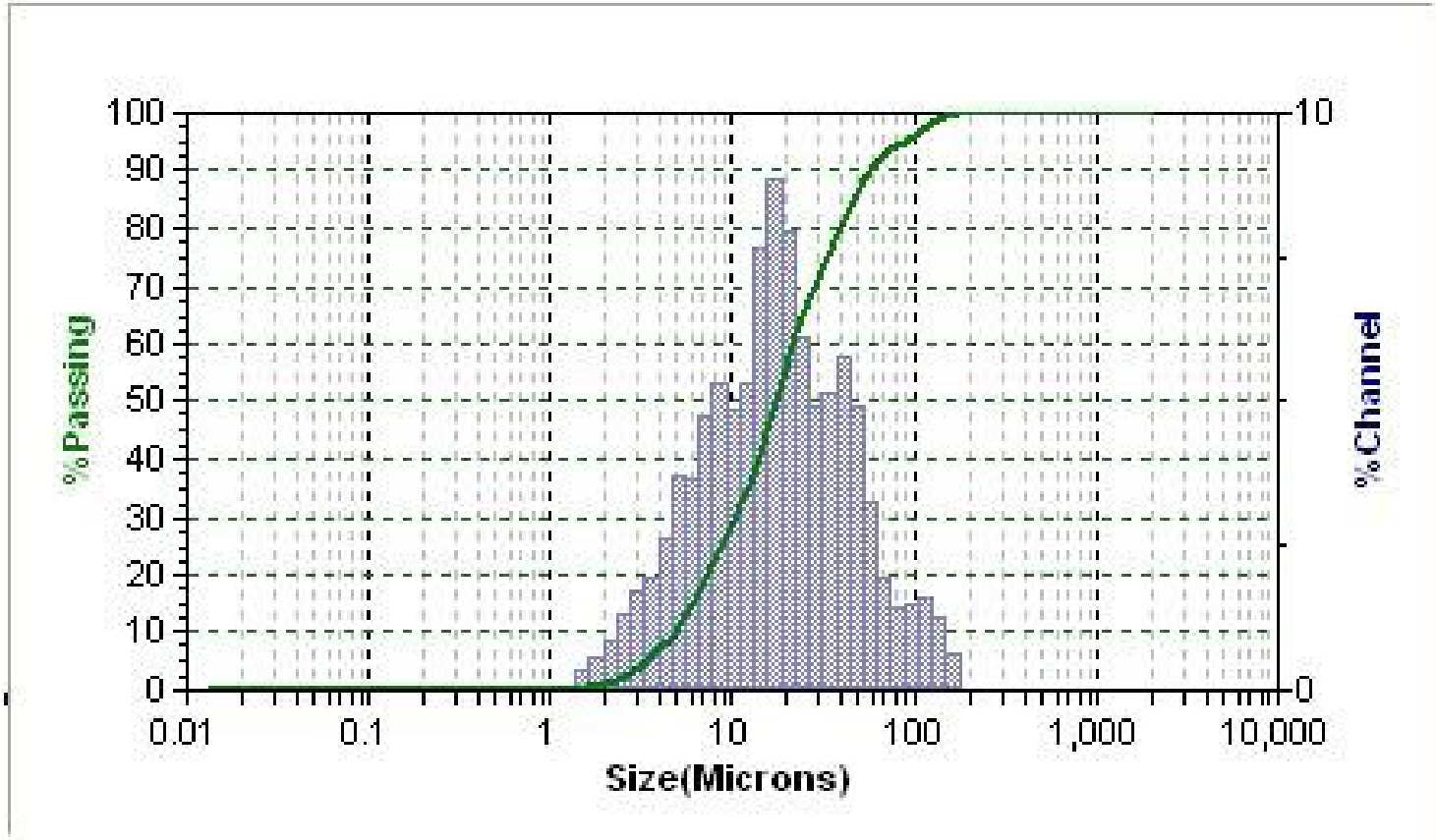


Figure: 15 d.

PARTICLE SIZE DISTRIBUTION F1

(SONICATION TIME= 3 MINUTES)

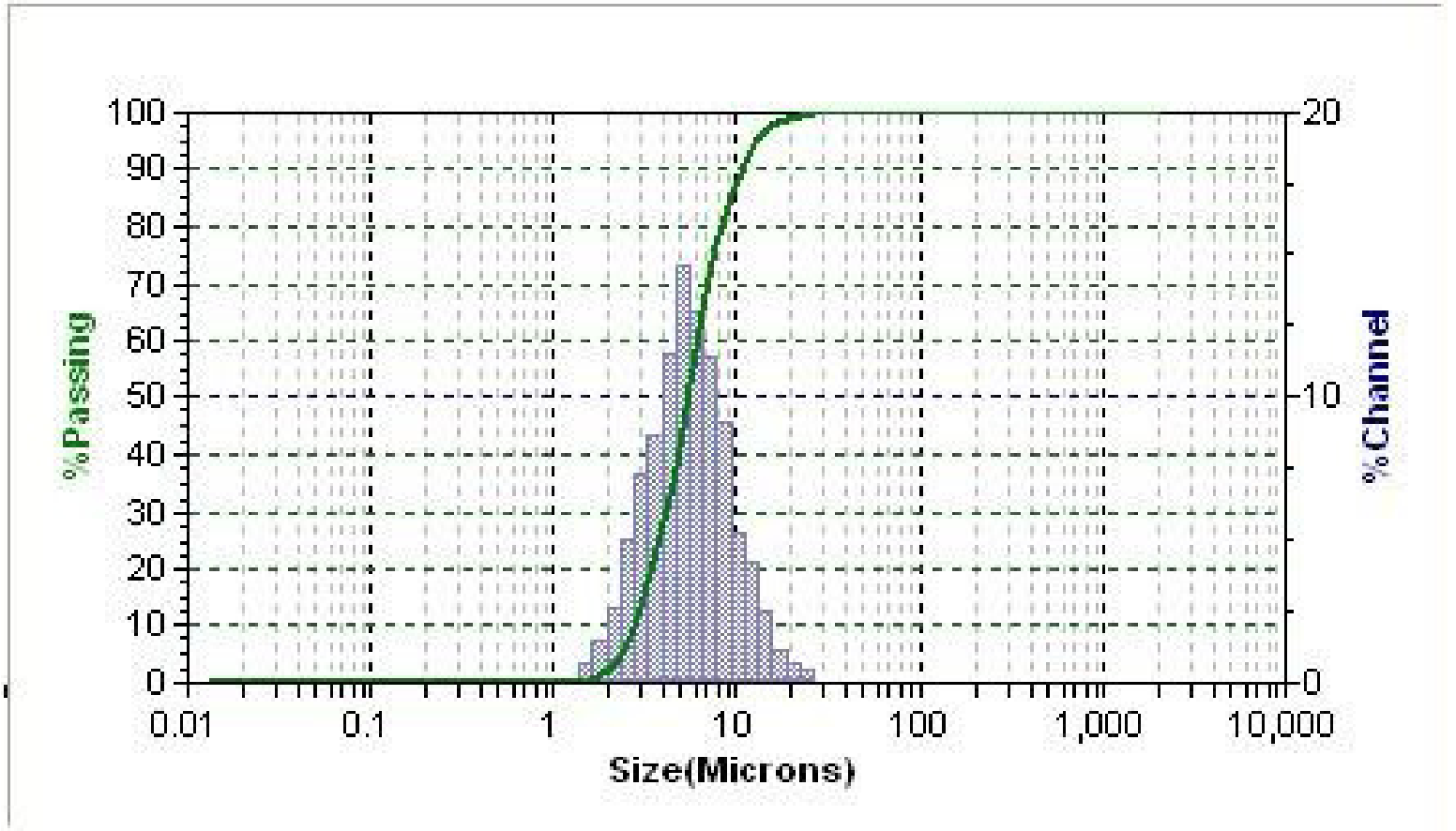


Figure: 15 e.

PARTICLE SIZE DISTRIBUTION F1

(SONICATION TIME= 4 MINUTES)

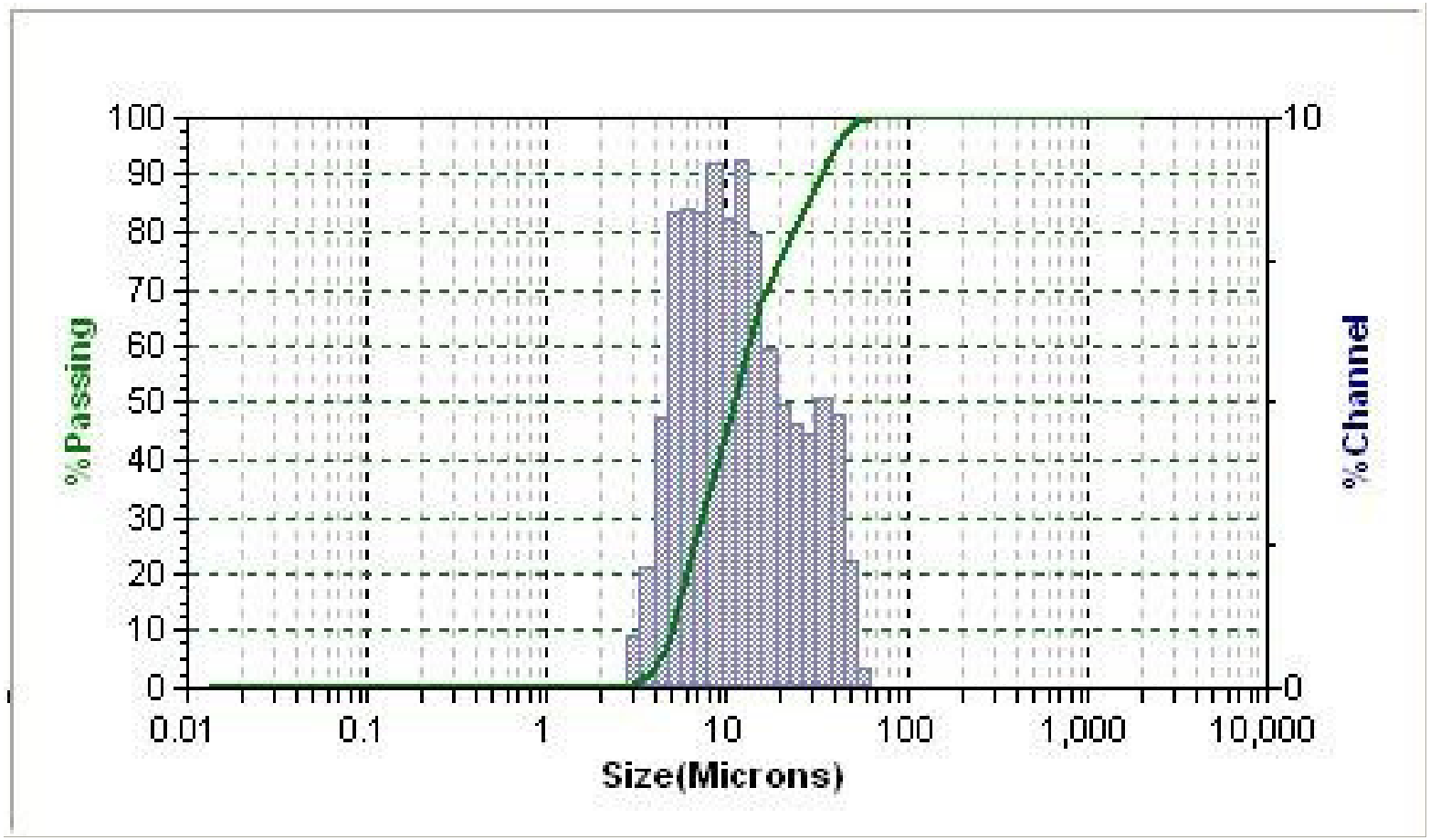
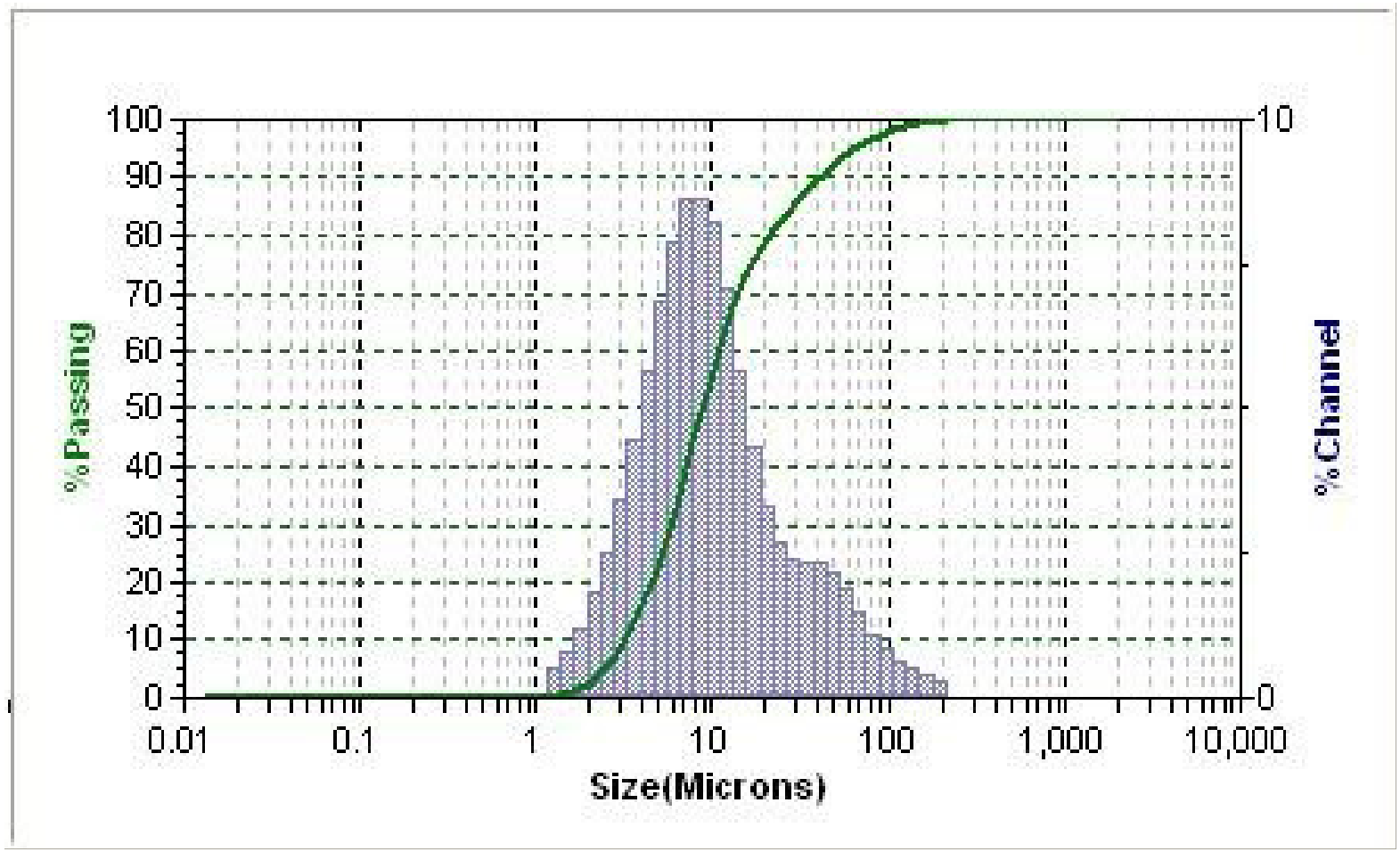


Figure: 15 f.

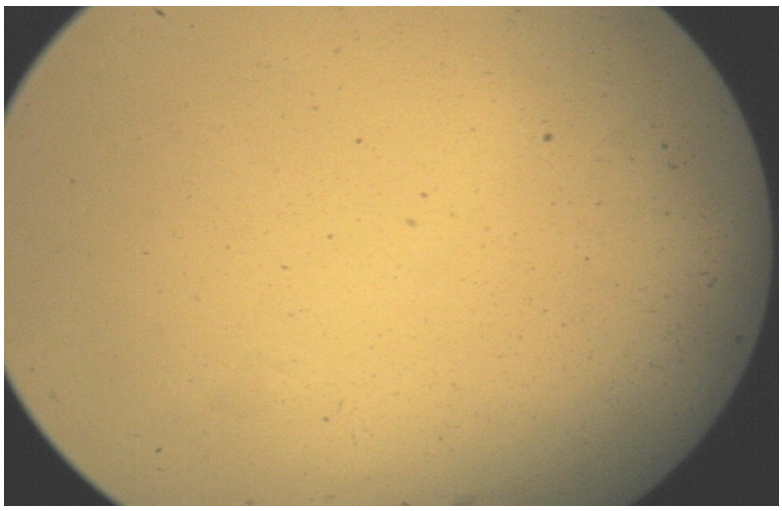
PARTICLE SIZE DISTRIBUTION F1

(SONICATION TIME= 5 MINUTES)

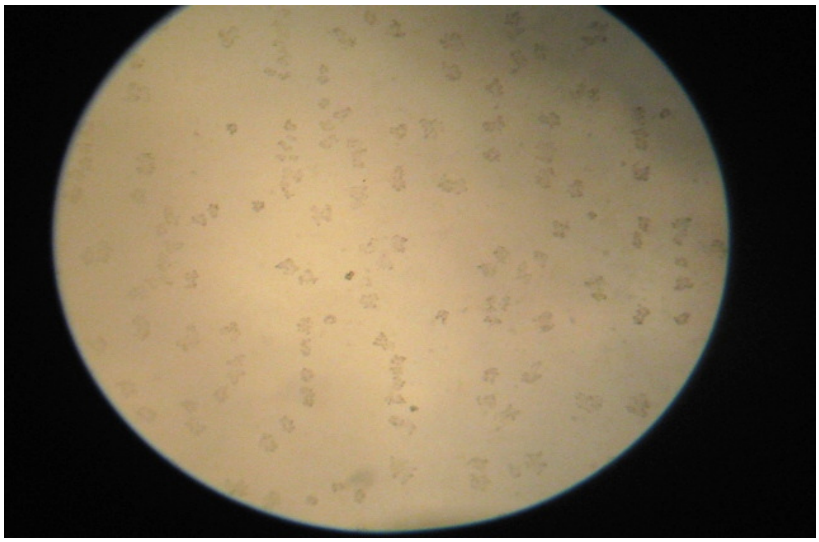


EFFECT OF OSMOTIC SHOCK

[A]



[B]



[C]

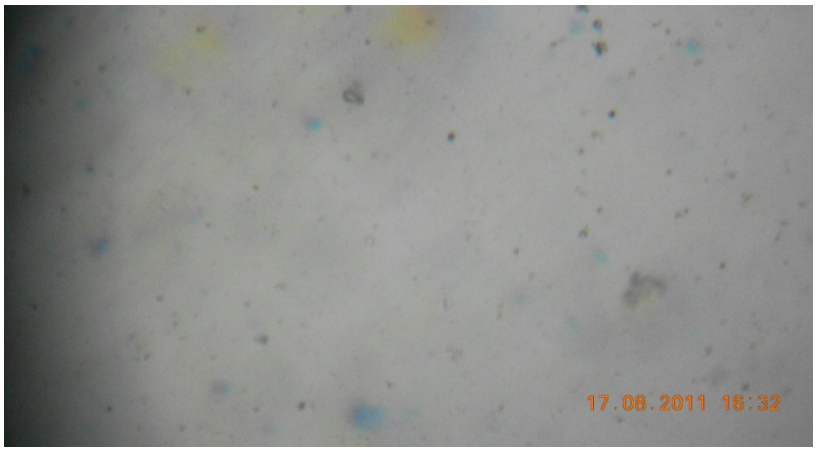
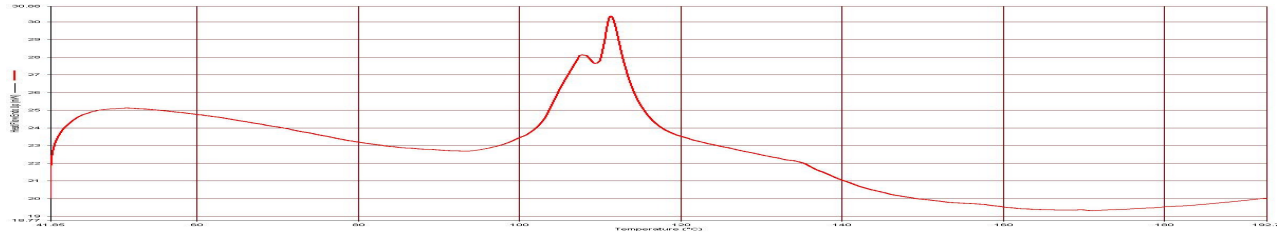


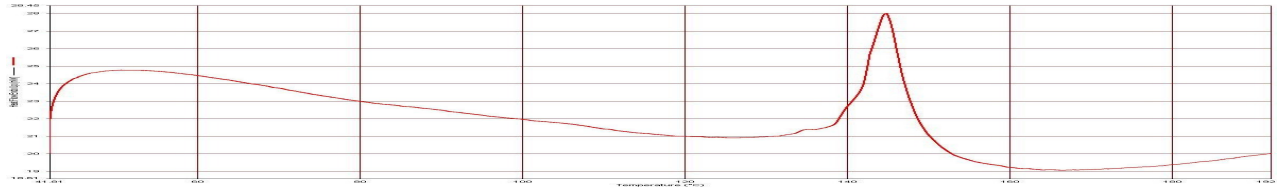
Figure: 16 [A] - HYPERTONIC (1.6% NaCl) [B]- HYPOTONIC (0.5% NaCl)

[C]- ISOTONIC (0.9% NaCl)

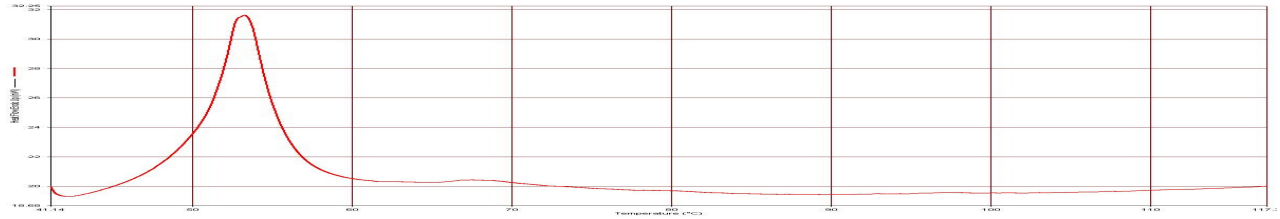
A)



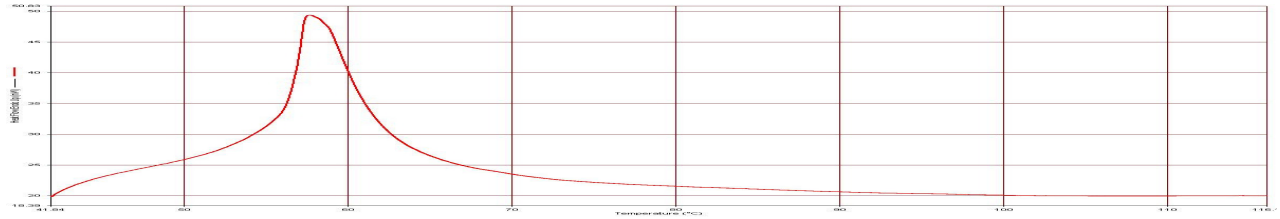
B)



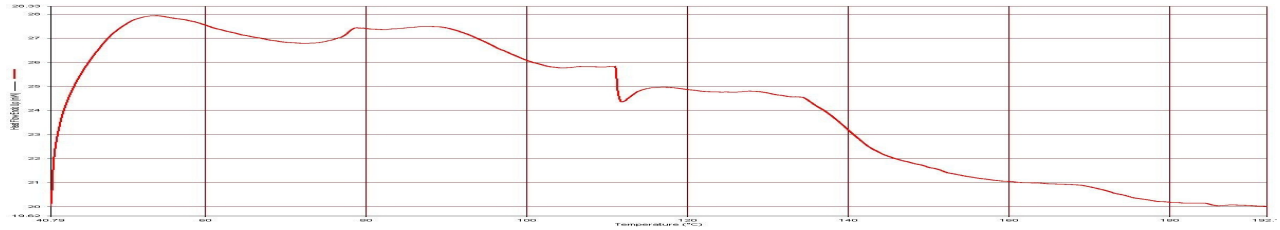
C)



D)



E)



F)

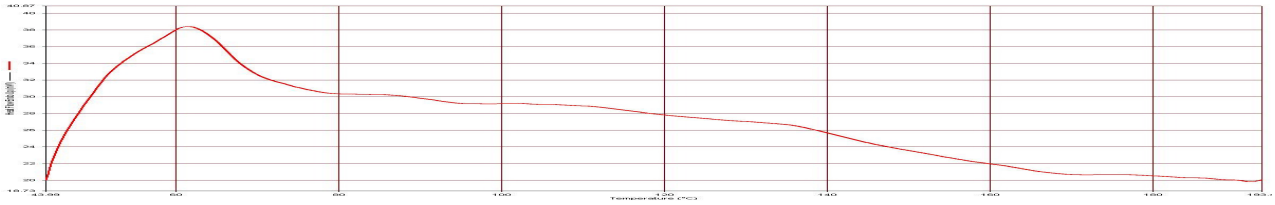


Figure: 28. DSC thermograms are as follows, A) Ramipril; B) Cholesterol; C) Span 40; D) Span 60; E) Formulation containing Span 40 and F) Formulation containing Span 60.

Figure : 20 a.

Particle size distribution (F12) at Malvern zeta sizer.

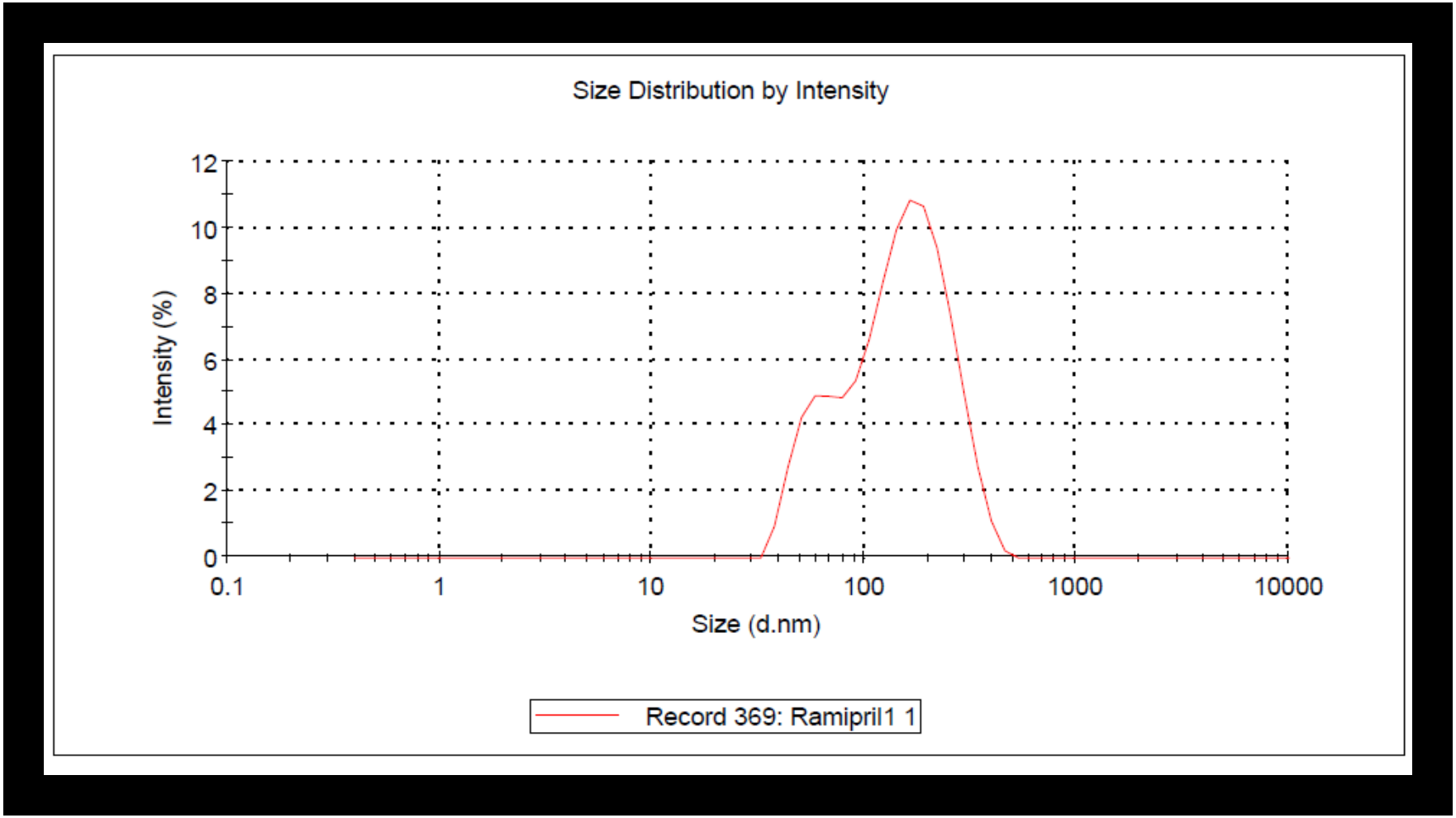


Figure : 20 b.

Particle size distribution (F12) at Malvern zeta sizer.

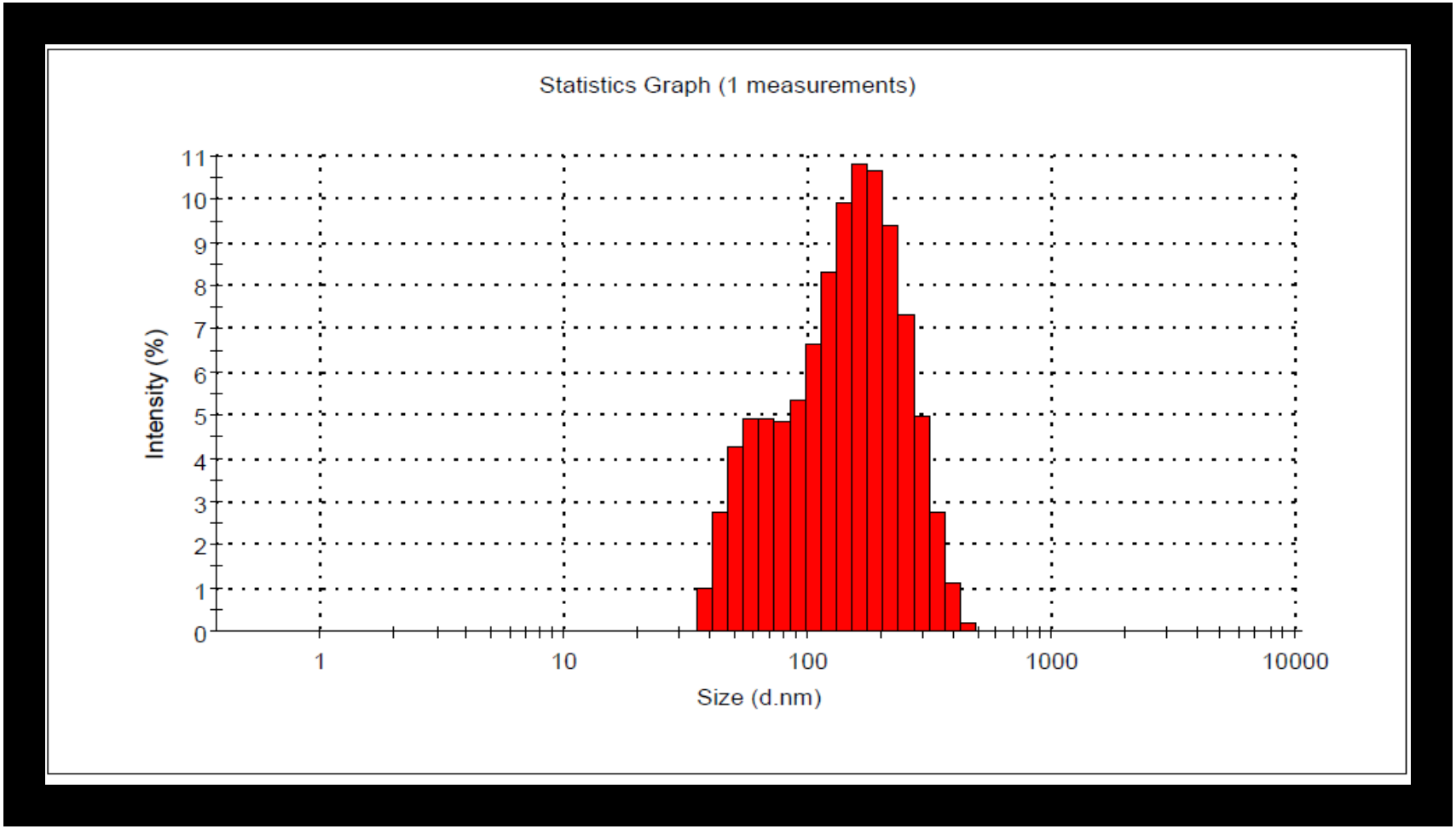


Figure : 21.

Zeta potential mesurement (F12) at Malvern zeta sizer

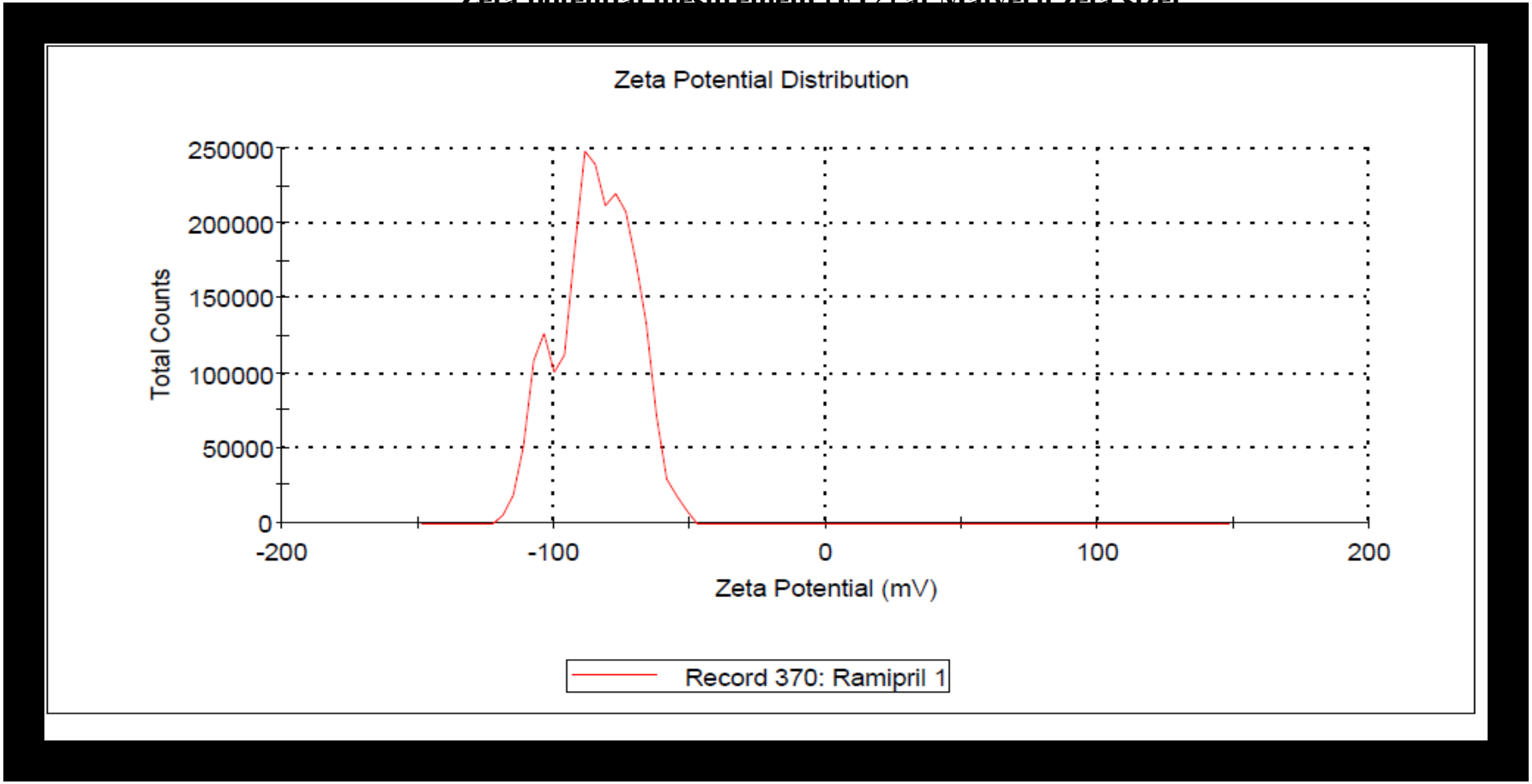


Figure: 22

SEM Photograph of F1

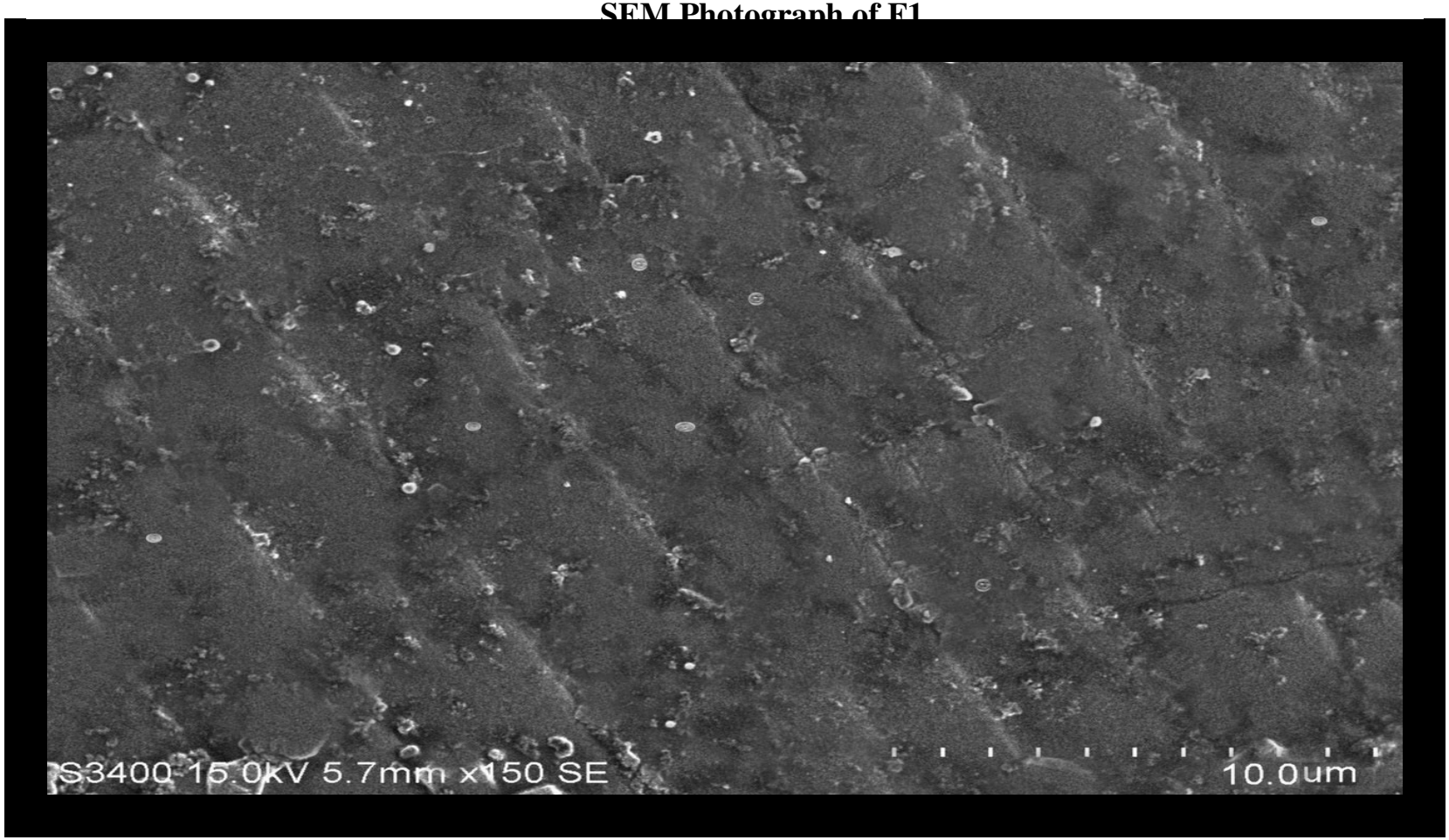


Figure: 23. FT-IR SPECTROSCOPY OF PURE DRUG- RAMIPRIL.

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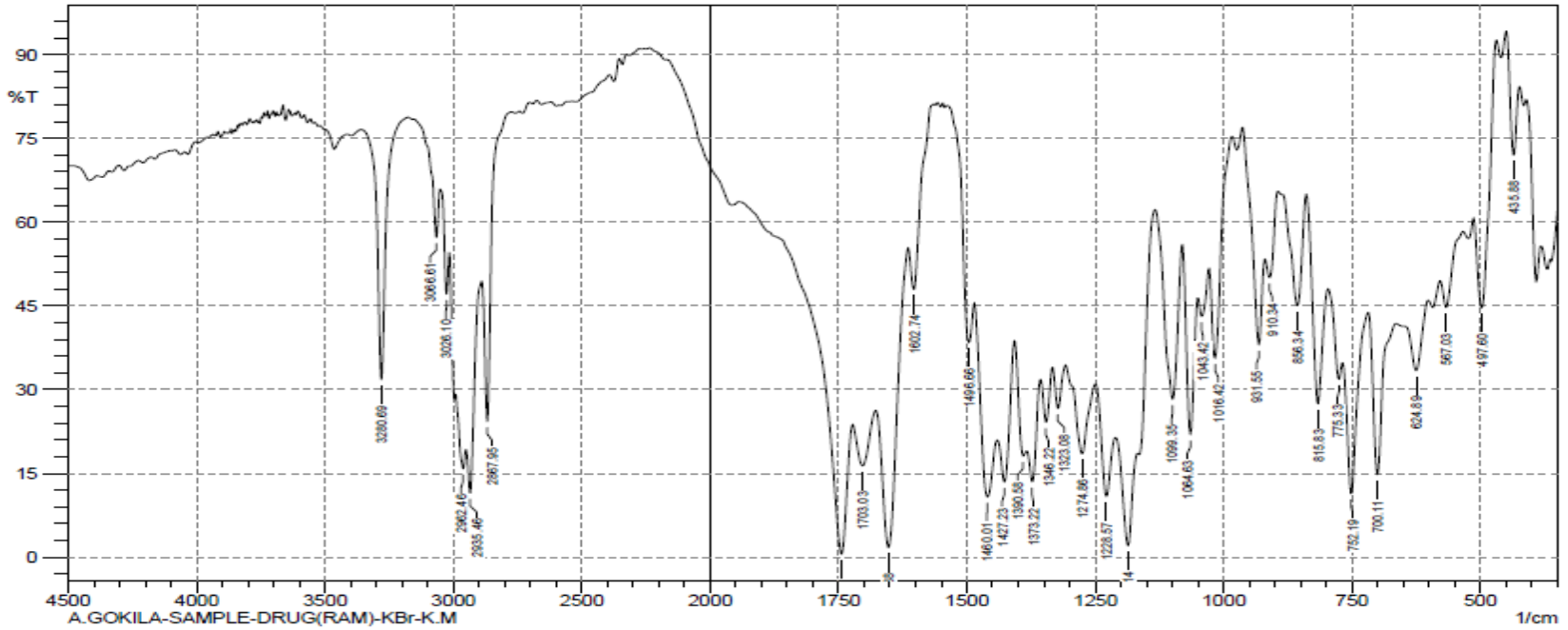
SHIMADZU



A.GOKILA-SAMPLE-DRUG(RAM)-KBr-K.M

Comment;

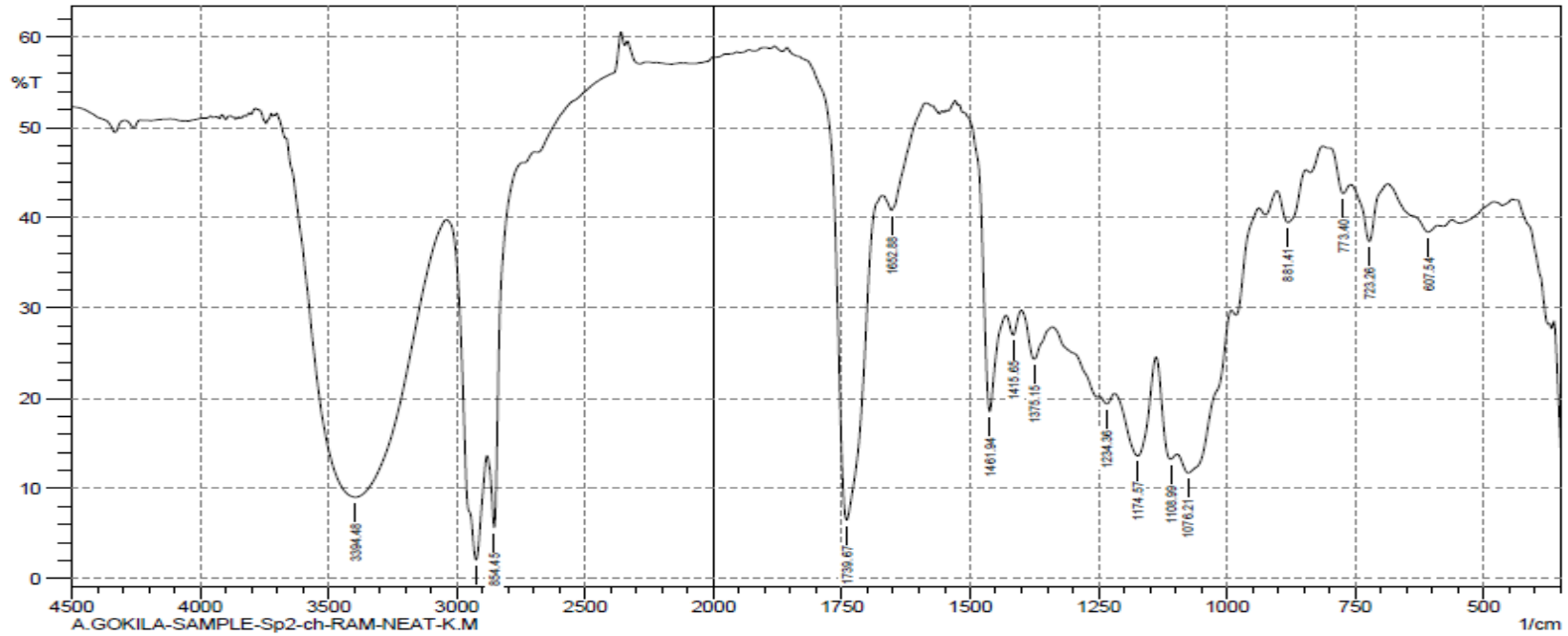
Resolution;
Apodization;

Date/Time; 7/13/2011 2:56:09 PM
User; USIC

Figure: 24a. FT-IR SPECTROSCOPY OF FORMULATION CONTAINING SPAN 20.

USIC-MKU

SHIMADZU



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Date/Time; 7/13/2011 3:17:45 PM
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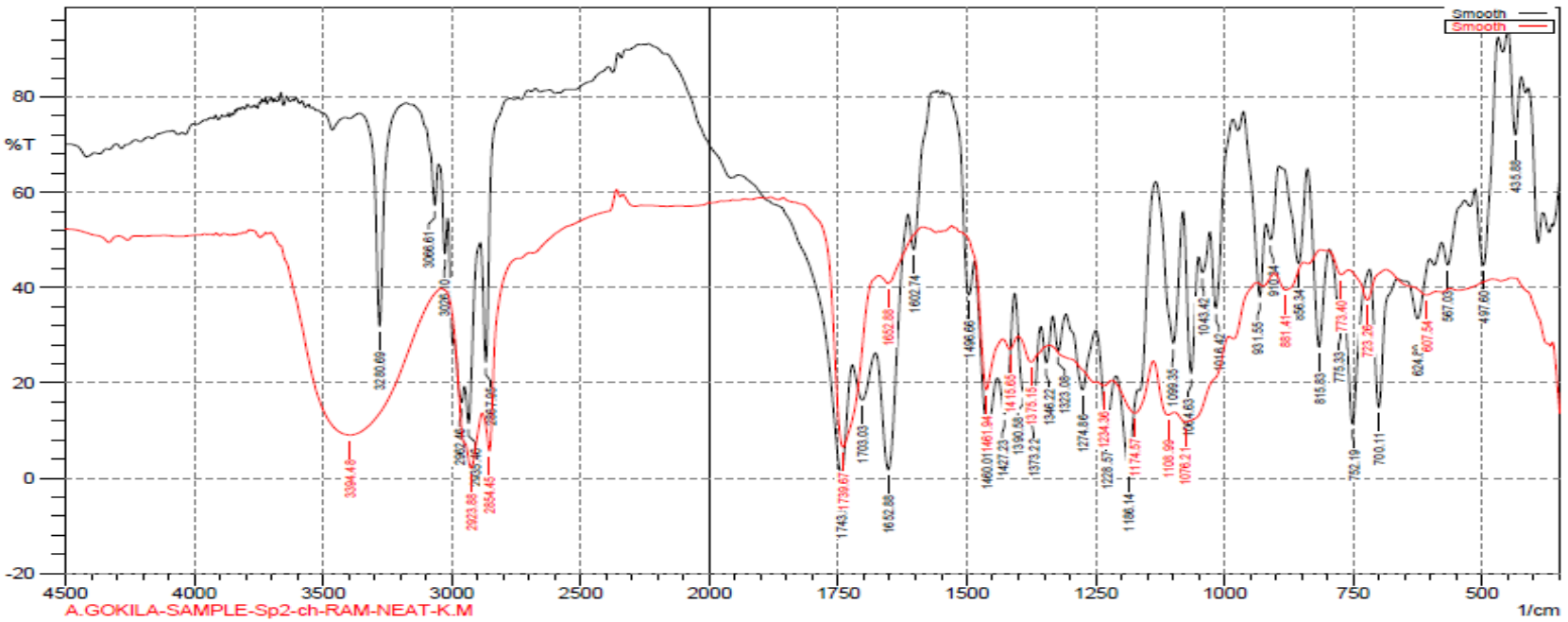
Figure: 24b.COMPARISON OF FT-IR SPECTROSCOPY OF FORMULATION CONTAINING SPAN 20 WITH RAMIPRIL.

USIC-MKU

SHIMADZU



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gure: 25a. FT-IR SPECTROSCOPY OF FORMULATION CONTAINING SPAN 40

USIC-MKU

SHIMADZU

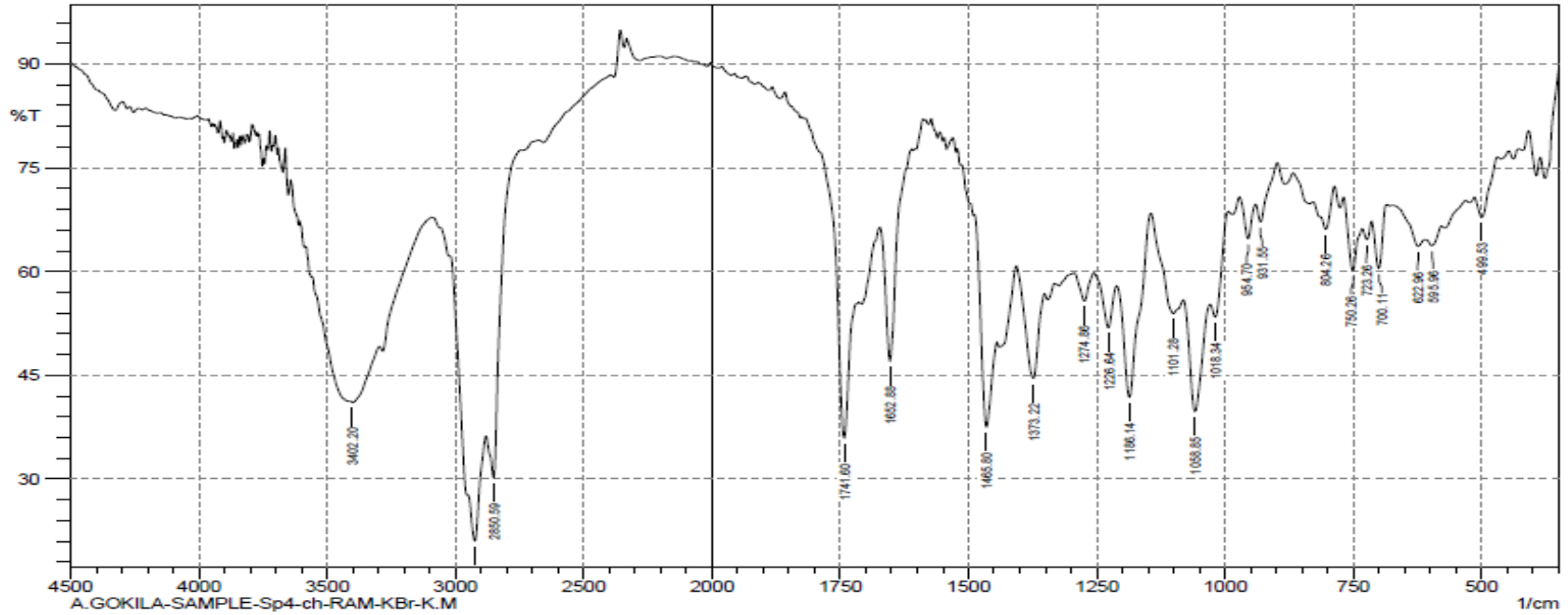

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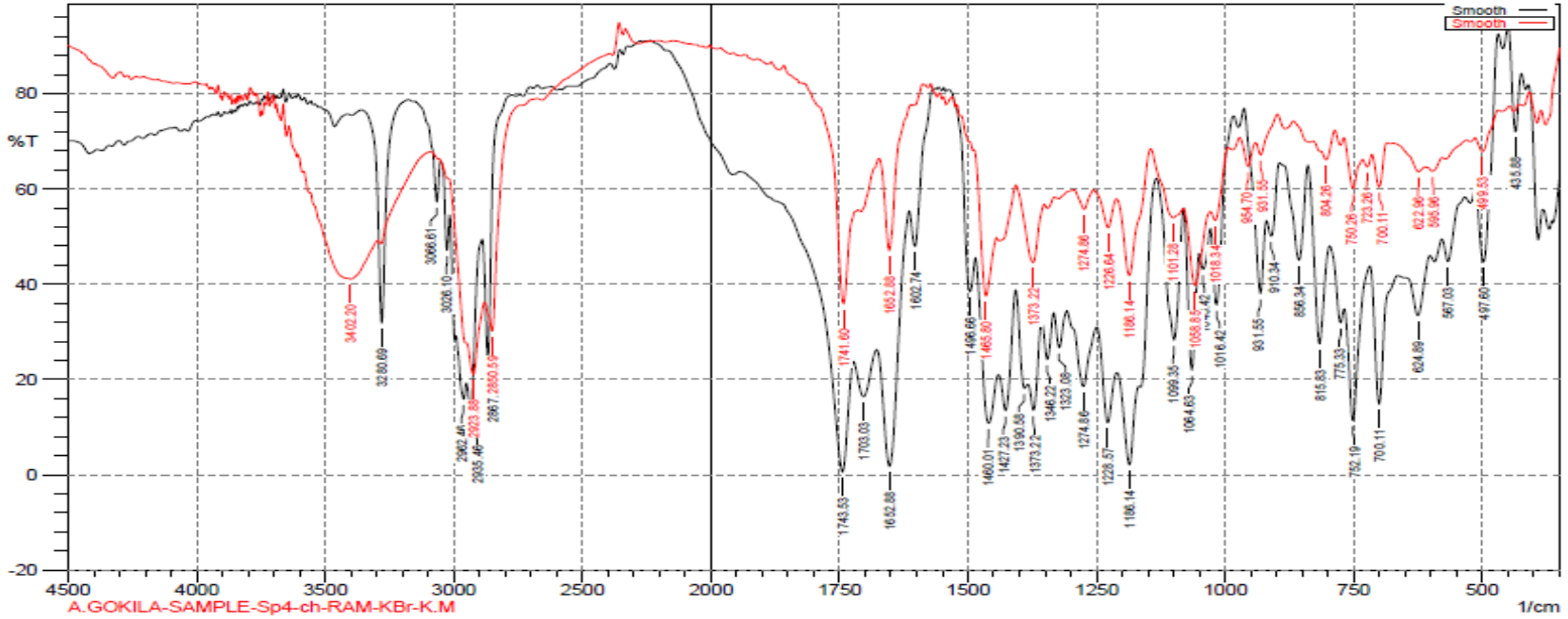
Figure: 25b. COMPARISON OF FT-IR SPECTROSCOPY OF FORMULATION CONTAINING SPAN 40 WITH RAMIPRIL.

USIC-MKU

SHIMADZU



A.GOKILA-SAMPLE-Sp4-ch-RAM-KBr-K.M

Comment;

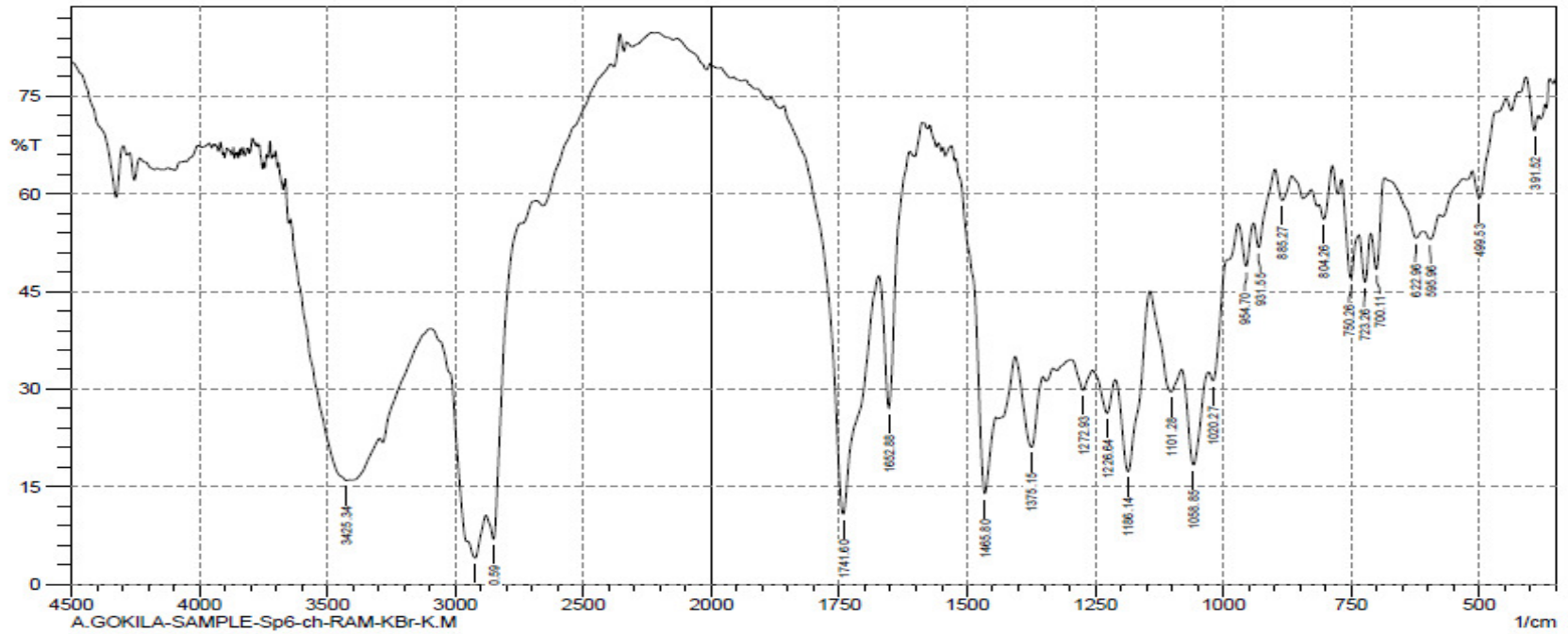
Resolution;
Apodization;

Date/Time; 7/13/2011 2:56:09 PM
User; USIC

Figure: 26a. FT-IR SPECTROSCOPY OF FORMULATION CONTAINING SPAN 60.

USIC-MKU

SHIMADZU



Comment;

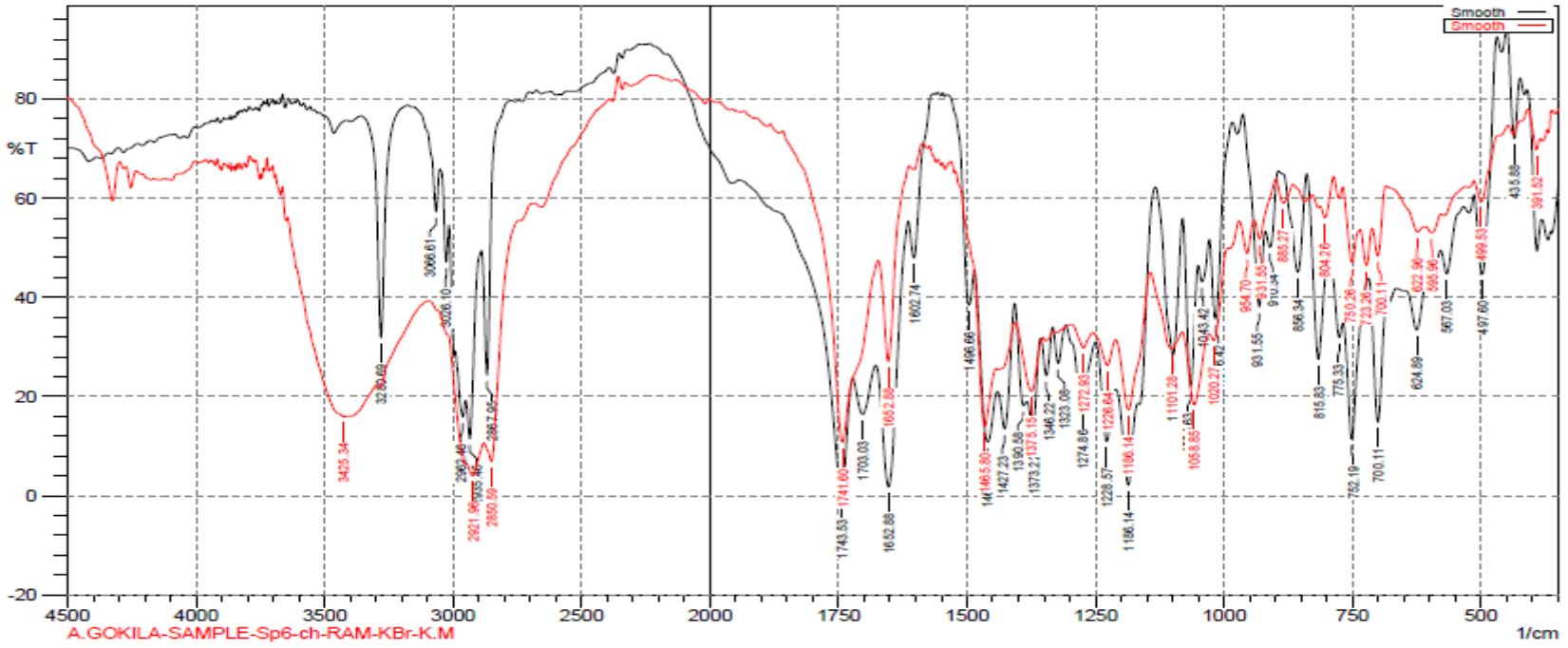
Resolution;
Apodization;

Date/Time; 7/13/2011 3:39:51 PM
User; USIC

Figure: 26b. COMPARISON OF FT-IR SPECTROSCOPY OF FORMULATION CONTAINING SPAN 60 WITH RAMIPRIL.

USIC-MKU

SHIMADZU



A.GOKILA-SAMPLE-Sp6-ch-RAM-KBr-K.M

Comment;

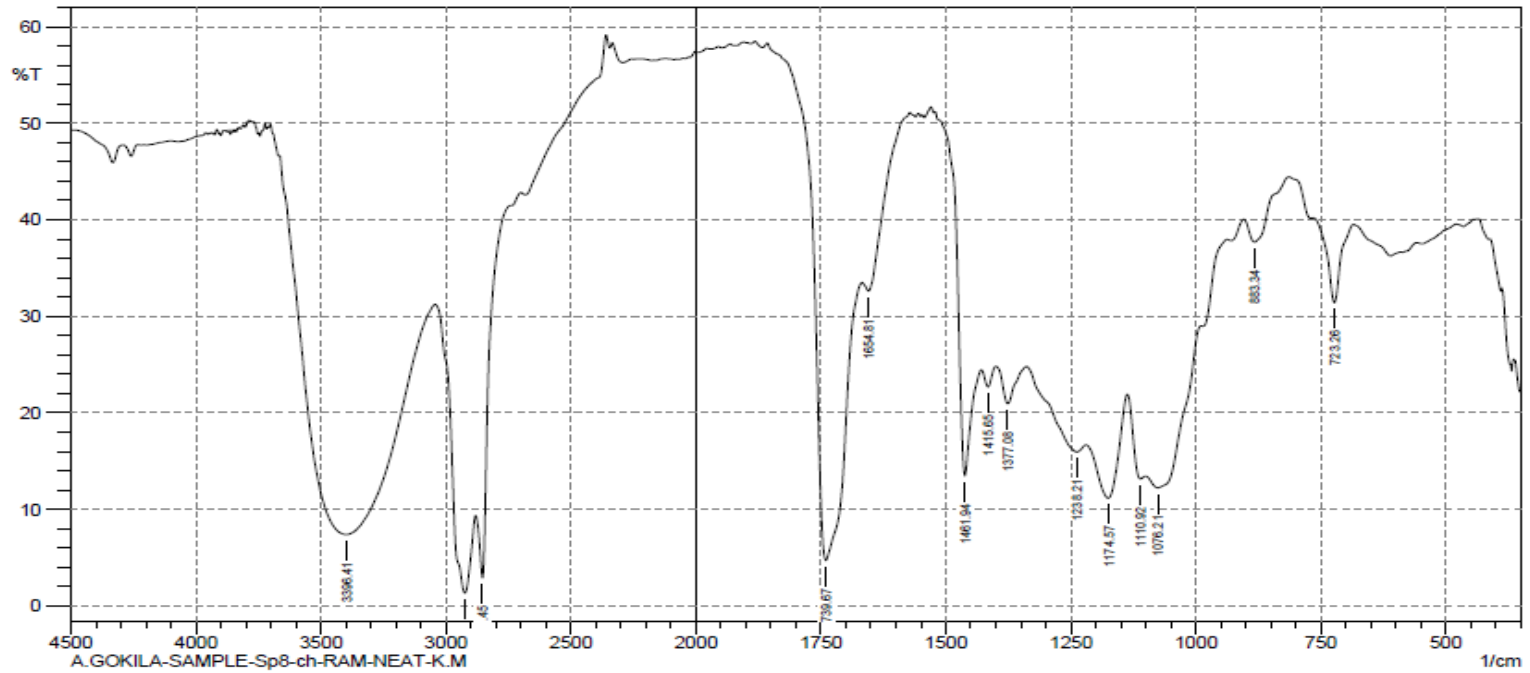
Resolution;
Apodization;

Date/Time; 7/13/2011 2:56:09 PM
User; USIC

Figure: 27a. FT-IR SPECTROSCOPY OF FORMULATION CONTAINING SPAN 80.

USIC-MKU

SHIMADZU



Comment;

Resolution;
Apodization;

Date/Time; 7/13/2011 3:49:46 PM
User; USIC

Figure: 27b. COMPARISON OF FT-IR SPECTROSCOPY OF FORMULATION CONTAINING SPAN 80 WITH RAMIPRIL.

USIC-MKU

SHIMADZU

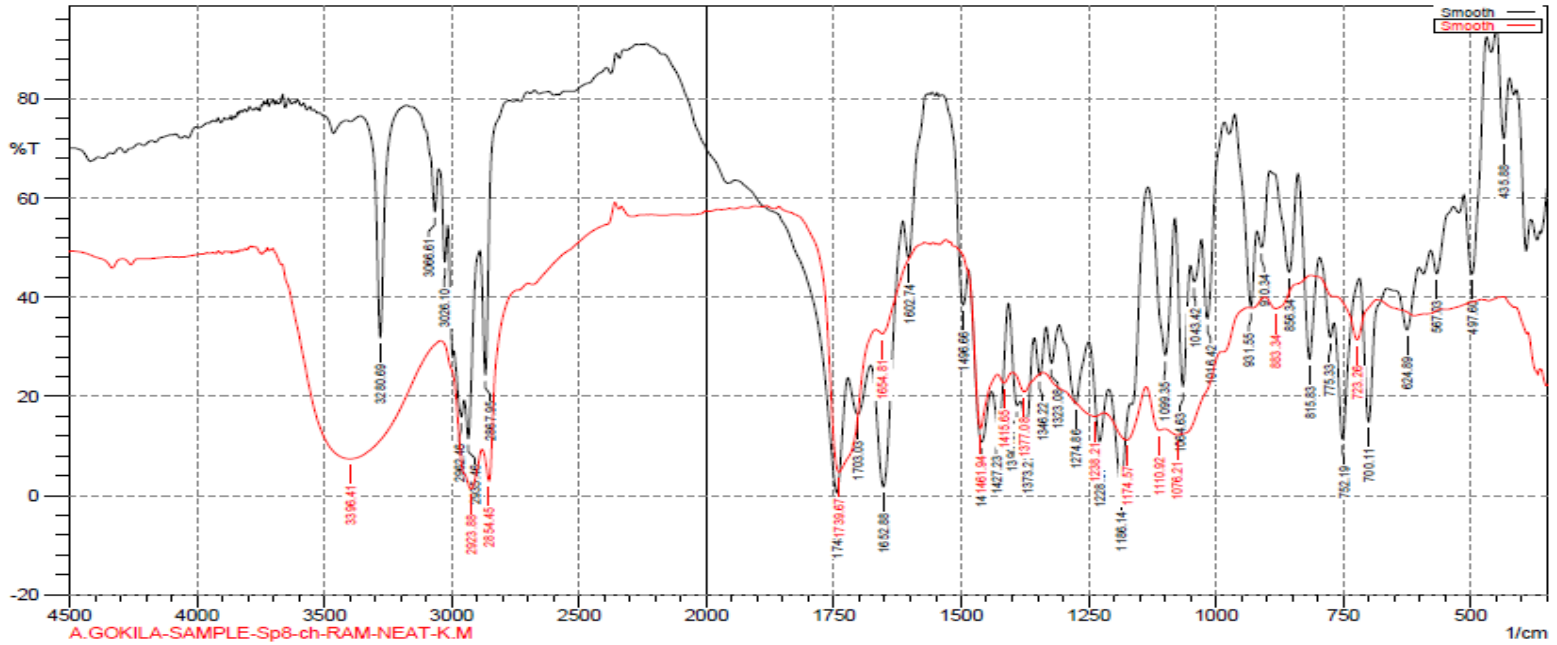


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Figure- 14

COMPARISON OF INVITRO RELEASE OF RAMIPRIL NIOSOMES CONTAINING DIFFERENT SURFACTANTS AT 1:1 RATIO.

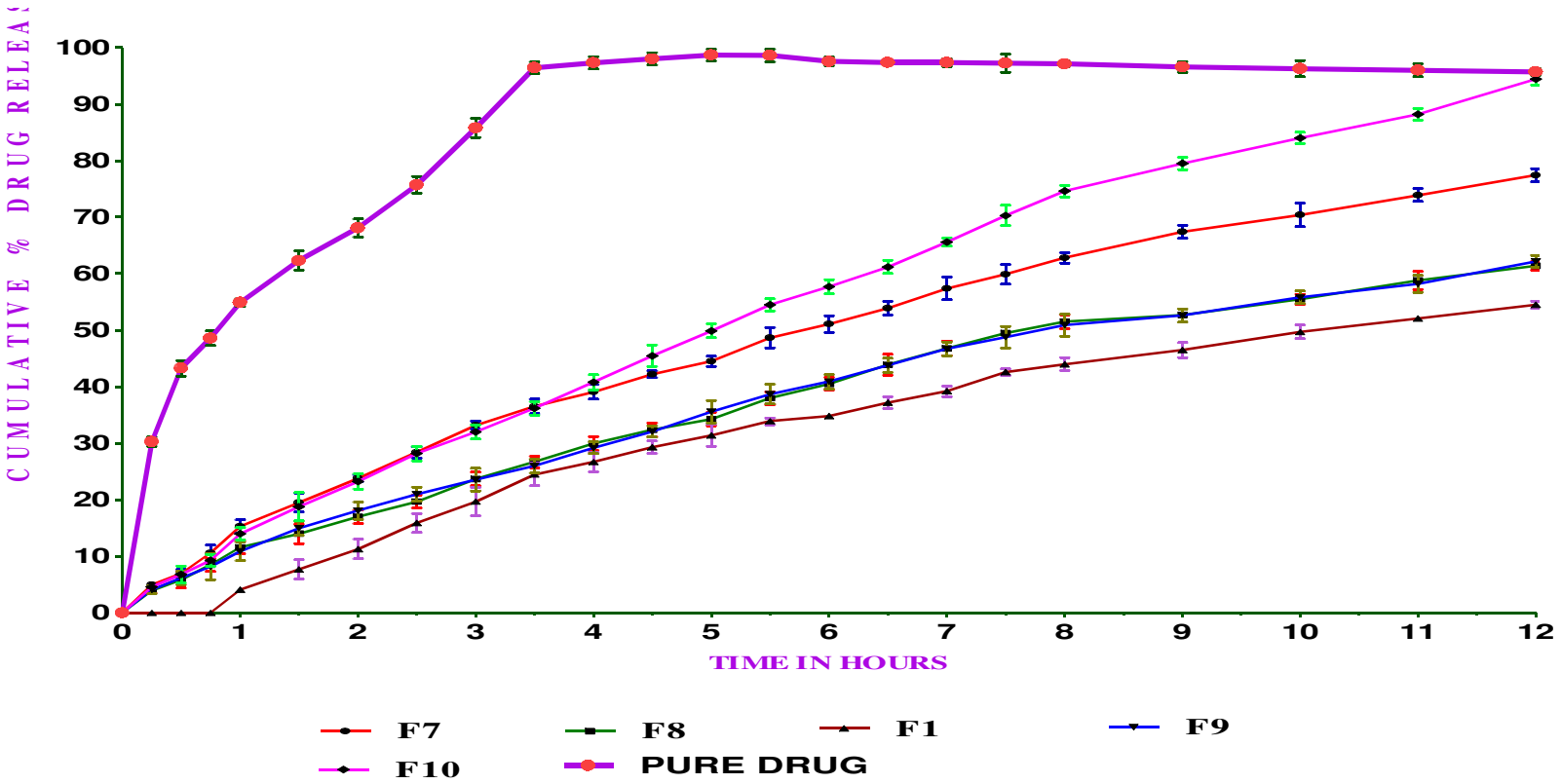


Figure- 17

COMPARISON OF INVITRO RELEASE OF RAMIPRIL NIOSOME SPAN 60 1:1 WITH AND WITHOUT CHARGE INDUCING AGENTS.

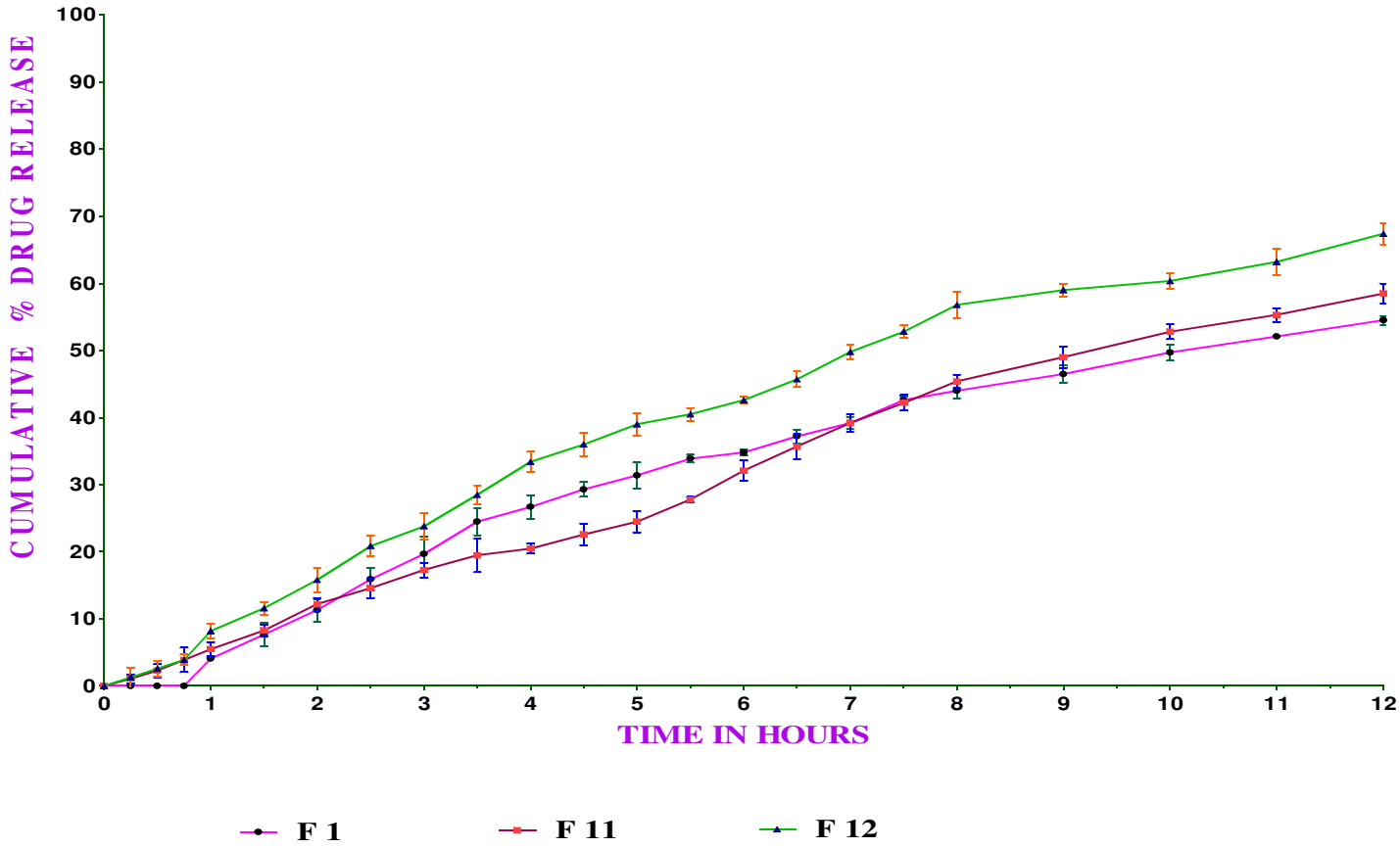

CUMULATIVE % DRUG RELEASE
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
7
8
9
10
11
12
TIME IN HOURS
F 1
F 11
F 12

Figure - 18

COMPARISON OF INVITRO RELEASE OF RAMIPRIL DRUG SOLUTION, NIOSOME F1 (SPAN 60 1:1) AND F12 (WITH DCP).

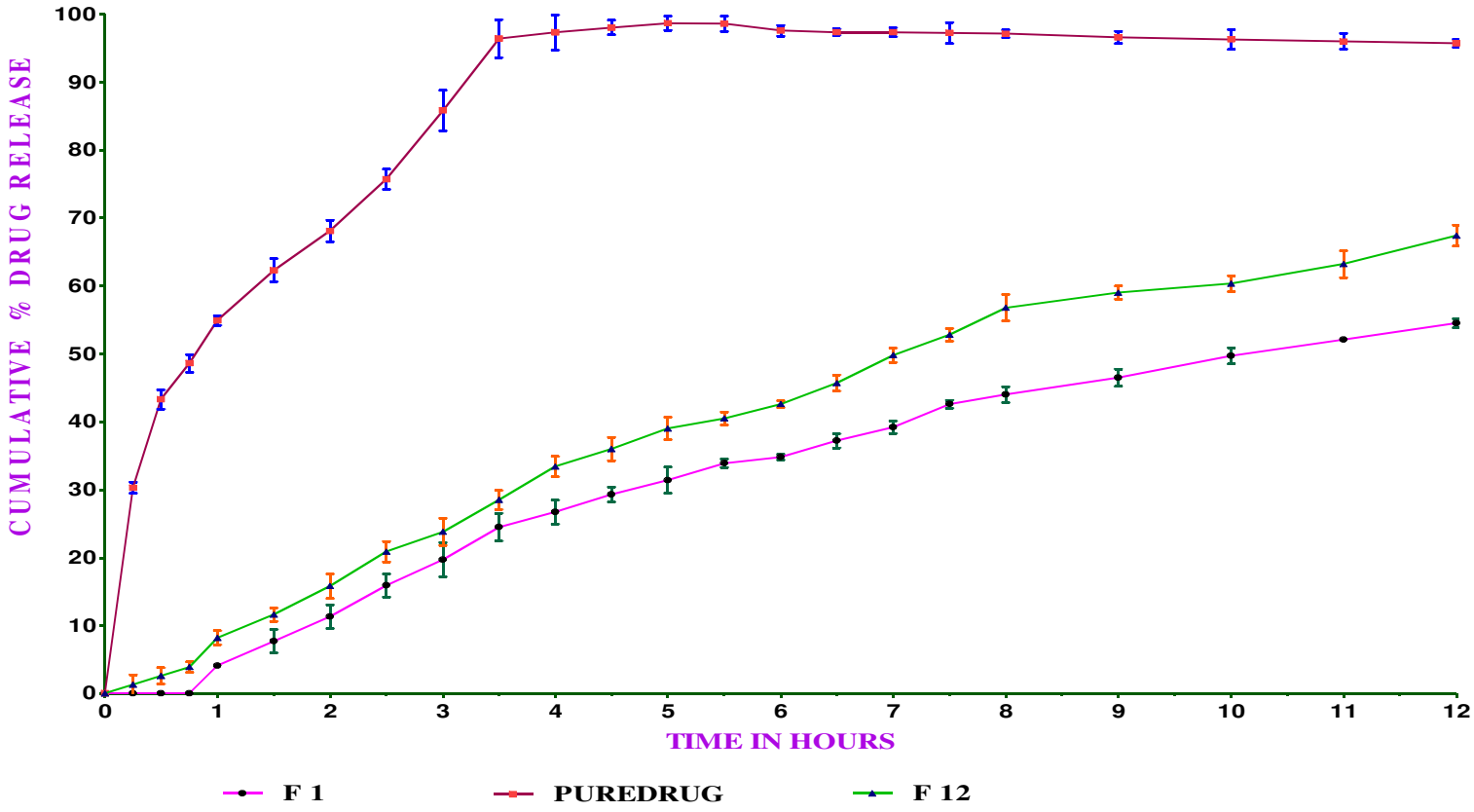
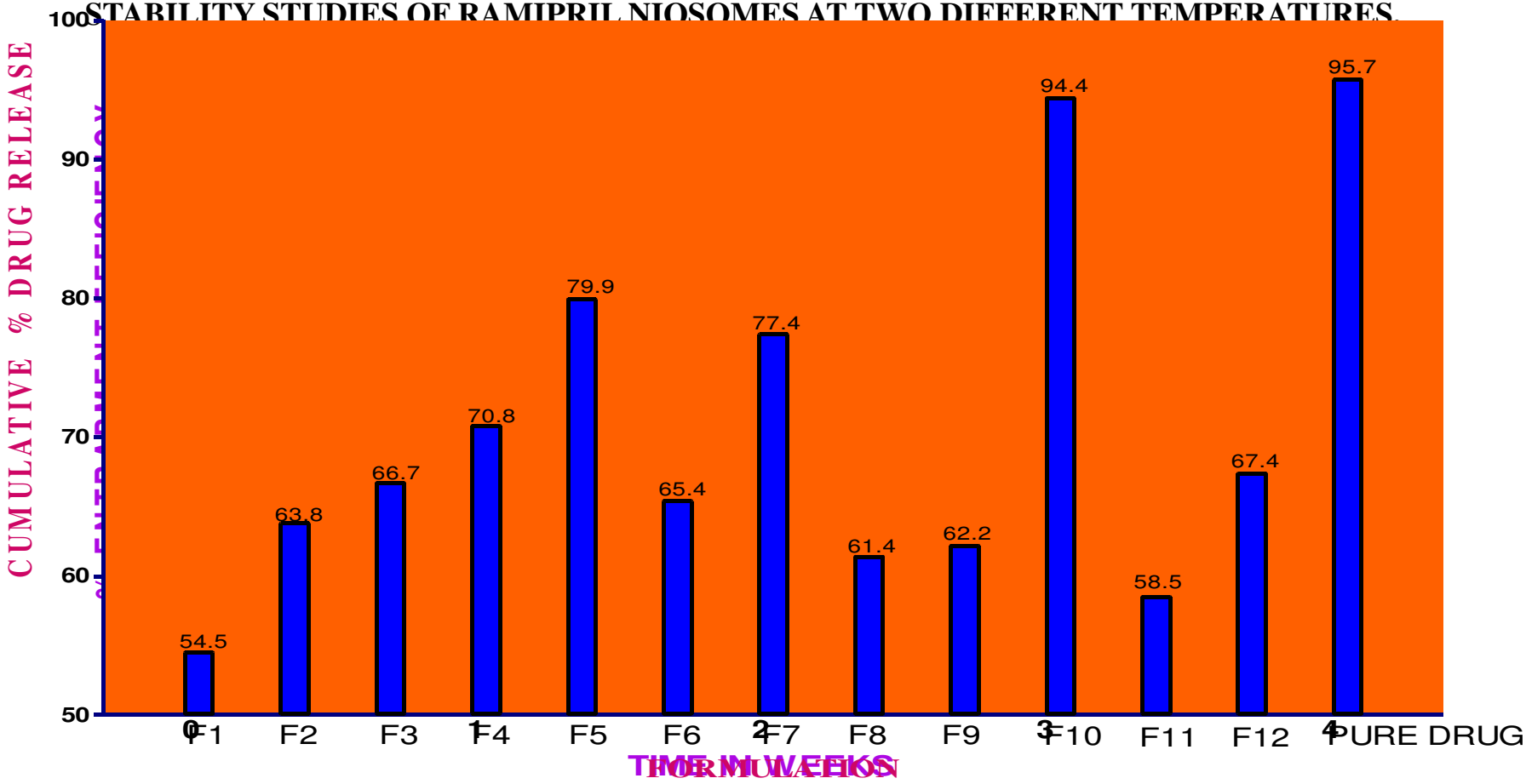


Figure - 19

COMPARISON OF INVITRO RELEASE STUDIES OF RAMIPRIL FORMULATIONS

Figure : 29.



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